SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF MODIFIED STEROIDS

ABSTRACT
OF THE
THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN
CHEMISTRY

BY

ASHRAF AHMED HASAN MASHRAI

UNDER THE SUPERVISION OF

Dr. SHAMSUZZAMAN

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)
2014

ABSTRACT

In the field of medicinal chemistry, steroids have been one of the most attractive areas for investigation due to their biological activities. Bioorganic chemists have reported a number of modifications in natural steroids resulting in the promotion of their activities. Even a small change in steroid moiety can elicit an extensive biological response. All these facts have attracted researcher to explore these after modifying them suitably to induce various pharmacological properties. This is still to be a fascinating field worldwide. Our laboratory is concerned mainly with the synthesis of steroidal compounds and their identification by chemical and spectral studies. a number of reports dealing with the preparation of modified steroids have appeared from our laboratory. The work embodied in this thesis describes the synthesis of modified steroids and their biological behavior. The identification of newly synthesized compounds has been ascertained by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. The whole work is divided into five chapters namely,

Chapter-1: Steroidal pyrazolines

Chapter-2: Steroidal pyranones

Chapter-3: Steroidal pyrazolones

Chapter-4: Steroidal thiazolidinones

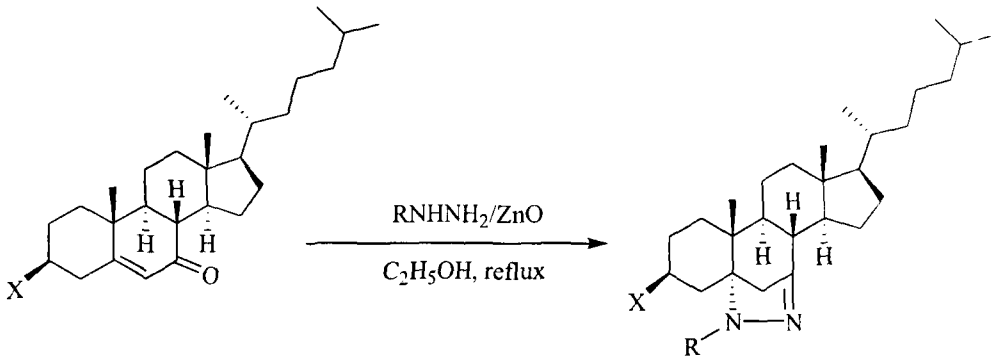
Chapter-5: Biological evaluation of newly synthesized compounds

The results are summarized below.

CHAPTER-1

Steroidal pyrazolines

Pyrazoline derivatives are important electron rich nitrogen heterocycles which play an important role in the diverse biological activities. These nitrogen heterocycles widely occur in nature in the form of alkaloids, vitamins, pigments and as constituents of plant and animal cells. Substituted pyrazolines have significant importance due to their wide spread pharmacological properties such as anticarcinogenic, antidiabetic, anti-inflammatory, DP-IV inhibitors, antitumor and antiparkinsonian. The above pharmaceutical interest prompted us to synthesize new substituted steroidal pyrazolines (**IV-IX**). The substrates selected for initial studies include cholest-5-en-7-one (**I**), 3β-acetoxycholest-5-en-7-one (**II**) and 3β-chlorocholest-5-en-7-one (**III**). To the best of our knowledge, this is the first report about the synthesis of steroidal pyrazolines using nano metal oxide as a heterogeneous catalyst. The products obtained have been characterized on the basis of their spectral (IR, ^{1}H NMR, ^{13}C NMR, MS) studies and elemental analysis.



(I-III)		(IV-IX)				
X		X	R		R	
H	(I)	H	H	(IV)	Ph	(VII)
OAc	(II)	OAc	H	(V)	Ph	(VIII)
Cl	(III)	Cl	H	(VI)	Ph	(IX)

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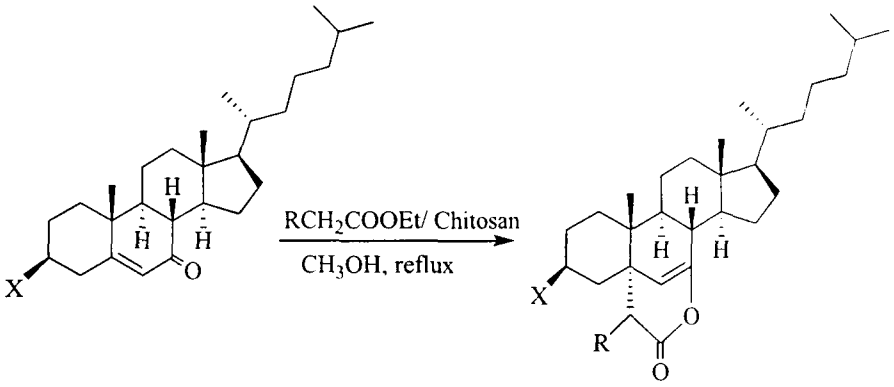
CHAPTER-2

Steroidal pyranones

It is known that many pyranone derivatives demonstrate a wide spectrum of pharmacological and biological activities. Various natural products also contain pyranone nucleus. Due to their biological significance there is a need to develop a new protocol for the synthesis of these compounds. Furthermore the development of new methods with greater efficacy, convenient procedures and better yield is of interest. This prompted us to synthesize new steroidal pyranone derivatives (**X-XV**) using readily available cholest-5-en-7-one and its analogs (**I-III**) in the presence of chitosan as heterogeneous, basic and green catalyst. With the best of our knowledge there are no literature data available regarding the synthesis of steroidal pyranone derivatives using chitosan as a catalyst.



(I-III) **(X-XV)**

X		X	R		R	
H	**(I)**	H	Cl	**(X)**	CH_3CO	**(XIII)**
OAc	**(II)**	OAc	Cl	**(XI)**	CH_3CO	**(XIV)**
Cl	**(III)**	Cl	Cl	**(XII)**	CH_3CO	**(XV)**

Green synthesis and biological evaluation of steroidal 2H-pyrans as anticancer and antioxidant agents. Shamsuzzaman, **Ashraf Mashrai**, H. Khanam, M. Asif, A. Ali, A. Sherwani and M. Owais.
***Journal of King Saud University-Science,* 2013** (in press);
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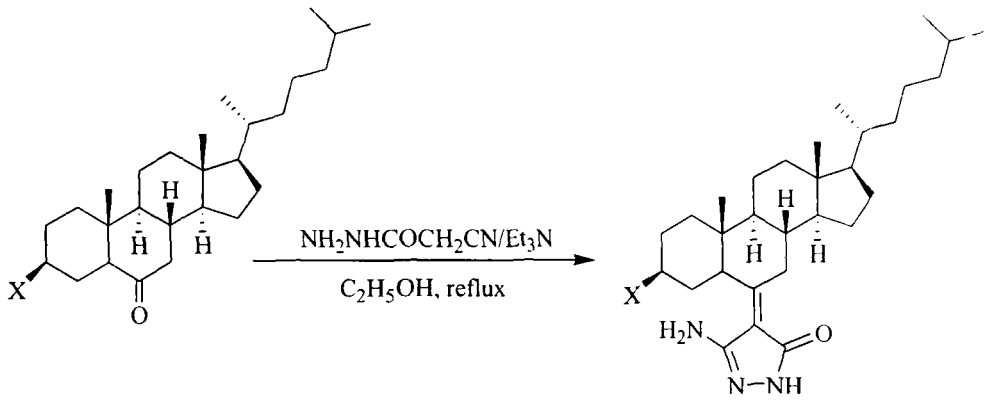
CHAPTER-3

Steroidal pyrazolones

In the recent past, the synthesis of pyrazolone derivatives have attracted attention of organic chemists because of the interesting physiological activity and profound endocrinological interest associated with them. With this realization, some papers appeared dealing with the synthesis of pyrazolones. This prompted us to undertake the work in this area. We subjected some easily available steroidal ketones (**XVI-XVIII**) to the reaction with cyanoacetohydrazide ($CNCH_2CONHNH_2$) and obtained 6-(5′-Amino-3′-oxo-dihydro-4*H*-pyrazol-4′-ylidine)-5α-cholestane (**XIX**), 3β-acetoxy-6-(5′-amino-3′-oxo-dihydro-4*H*-pyrazol-4′-ylidine)-5α-cholestane (**XX**) and 3β-chloro-6-(5′-amino-3′-oxo-dihydro-4*H*-pyrazol-4′-ylidine)-5α-cholestane (**XXI**) respectively. The products obtained have been characterized on the basis of their spectral (IR, ^{1}H NMR, ^{13}C NMR and MS) studies and elemental analysis.



(XVI-XVIII)		(XIX-XXI)	
X		X	
H	(**XVI**)	H	(**XIX**)
OAc	(**XVII**)	OAc	(**XX**)
Cl	(**XVIII**)	Cl	(**XXI**)

Synthesis, evaluation and docking studies on steroidal pyrazolones as anticancer andantimicrobial agents. Shamsuzzaman, **Ashraf Mashrai**, A. Ahmad, A. M. Dar, H. Khanam, M. Danishuddin and A. U. Khan
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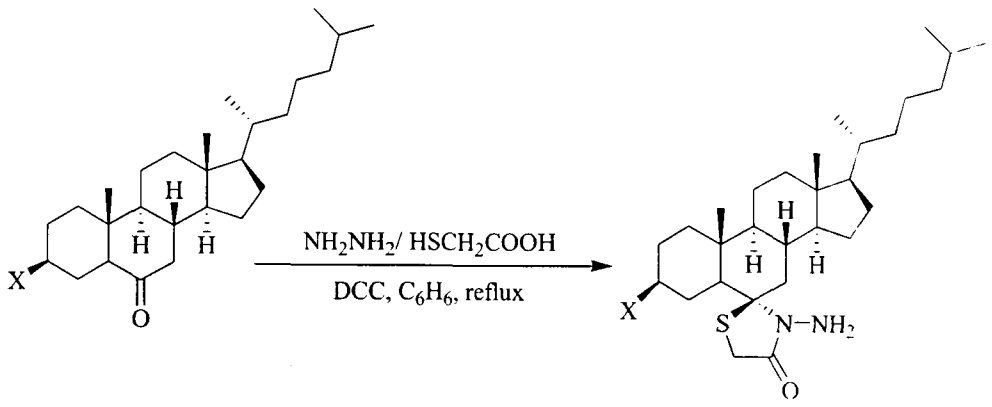
CHAPTER-4

Steroidal thiazolidinones

In recent years, thiazolidinones are the most extensively investigated class of compounds. Such type of compounds have many interesting activity profiles namely COX-1 inhibitors, inhibitors of the bacterial enzyme MurB, non-nucleoside inhibitors of HIV-RT and antihistaminic agents. Consequently, many different protocols have been developed that allow the synthesis of thiazolidinone skeleton. These methods employ a one-pot three-component condensation or a two-step synthesis. Motivated by these facts a successful attempt is made to synthesize steroidal thiazolidinones (**XXII-XXIV**) by a convenient one-pot method. For this purpose 5α-cholestan-6-one (**XVI**), 3β-acetoxy-5α-cholestan-6-one (**XVII**) and 3β-chloro-5α-cholestan-6-one (**XIII**) were allowed to react with mercaptoacetic acid and hydrazine hydrate in the presence of DCC. The structure of the products have been characterized on the basis of their spectral (IR, ^{1}H NMR, ^{13}C NMR and MS) studies and elemental analysis.



(XVI-XVIII)

X	
H	**(XVI)**
OAc	**(XVII)**
Cl	**(XVIII)**

(XXII-XXIV)

X	
H	**(XXII)**
OAc	**(XXIII)**
Cl	**(XXIV)**

CHAPTER-5

Biological evaluation of newly synthesized compounds

The global production of new drugs has increased in the last decades, and although many of the products have been beneficial for mankind, many of them are also toxic and can accumulate within organisms. In the global drug market, steroid drugs rank second only after antibiotics. Search of novel drugs is field of current and growing interest and many compounds have been synthesized to this aim. One possibility to achieve this goal is the combination of a steroid molecule with structural elements possessing appropriate biological activities. The advantage of employing hydrophobic steroid units is their ability to interact with cell membranes and thus pave the way for biological activity of such hybrid molecules. To gain insight on how modifications on ring-B in steroids nucleus can affect the biological activity, different steroidal derivatives with a variety of functionalities attached to steroidal skeleton have been synthesized and evaluated *in silico* and *in vitro* for their biological activity. In the *in slico* studies, we studied the physicochemical parameters "Rule of Five" of the synthesized compounds and we calculated the bioactivity score for six criteria, GPCR ligand activity, ion channel modulation, kinase inhibition activity, protease inhibitor, enzyme inhibitor and nuclear receptor ligand activity. The cytotoxic potential of the synthesized compounds were studied against panel of selective human cancer cells by MTT assay. The panel of cancer cells encompassed **HepG2** (hepatocellular carcinoma), **A549** (lung adenocarcinoma epithelium), **SW480** (colon adenocarcinoma), **HeLa** (cervical carcinoma) and **HL-60** from (promyelocytic leukaemia). Doxorubicin and Cytarabine were used as cytotoxic drugs of reference. The compounds were also tested against one normal cell line **PBMC** (Blood peripheral mononuclear cell isolation). The newly synthesized compounds were screened against a variety of bacterial strains (*Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae*) by disk diffusion method. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 36 °C. The minimum inhibitory concentration (MIC) of all the compounds was determined. Ciprofloxacin was used as positive control, whereas DMSO poured disk was used as negative control and then minimum inhibitory

concentration (MIC) was evaluated by the macro-dilution test. The *in vitro* antifungal activities were carried out using (*Candida albicans, Aspergillus fumigatus, Trichophyton mentagrophytes* and *Penicillium marneffei*) by agar diffusion method. The minimum inhibitory concentration (MIC) and Inhibition zones were determined as in antibacterial activity. The activity of the synthesized compounds was compared with Fluconazole as positive control and DMSO as negative control. The synthesized compounds were also tested for antioxidant activities by 1,1diphenylpicrylhydrazyl (DPPH) method. GOLD (Genetic Optimization for Ligand Docking) 5.0 was used for docking of compounds **(XIX- XXIV)** against S12 and CYP 51 proteins.

LIST OF PUBLICATIONS

1. **Synthesis, evaluation and docking studies on steroidal pyrazolones as anticancer and antimicrobial agents**
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4. **Synthesis, growth, spectral, thermal and crystallographic studies of 5α,6α-epoxycholestane single crystals**
   Shamsuzzaman, Hena Khanam, **Ashraf Mashrai**, Musheer Ahmad, Yahia N. Mabkhot,Wolfgang Frey, Nazish Siddiqui.
   J. Cryst. Growth, 384 **(2013)** 135-143

5. **Synthesis and anti-tumor evaluation of B-ring substituted steroidal pyrazoline derivatives**
   Shamsuzzaman, Hena Khanam, **Ashraf Mashrai**, Asif Sherwani, Mohammad Owais,
   Nazish Siddiqui.
   Steroids, 78 **(2013)** 1263-1272

6. **Construction of novel steroidal isoxazolidinone derivatives under Vilsmeier-Haack conditions**
   Shamsuzzaman, Hena Khanam, **Ashraf Mashrai**, Nazish Siddiqui.
   Tetrahedron Lett. 54 **(2013)** 874-877

7. **6-Hydroxyimino-5α-cholestane**
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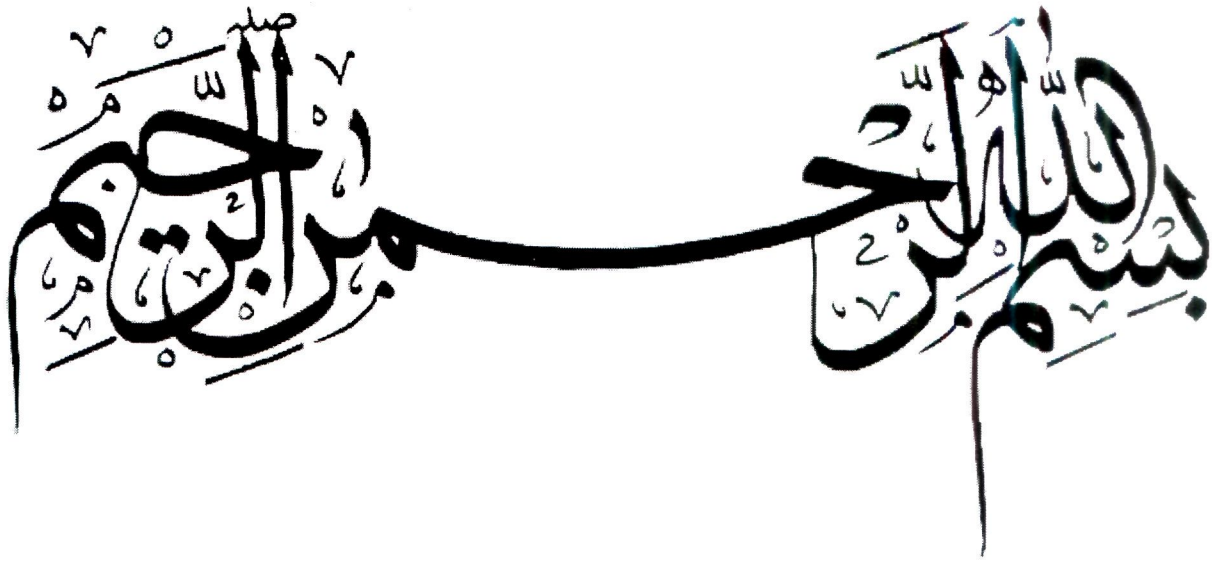
بسم الله الرحمن الرحيم

CANDIDATE'S DECLARATION

I, **Ashraf Ahmed Hasan Mashrai,** Department of **Chemistry** certify that the work embodied in this Ph.D thesis is my own bonafide work carried out by me under the supervision of **Prof. Shamsuzzaman** at Aligarh Muslim University, Aligarh. The matter embodied in this Ph.D thesis has not been submitted for the award of any other degree.

I declare that I have faithfully acknowledged, given credit to and referred to the research workers wherever their works have been cited in the text and the body of the thesis. I further certify that I have not willfully lifted up some other's work, para, text, data, result, etc. reported in the journals, books, magazines, reports, dissertations, theses, etc., or available at web-sites and included them in this Ph.D. thesis and cited as my own work.

Date: 31.3.2014

(Signature of the candidate)

Ashraf Ahmed Hasan Mashrai
(Name of the candidate)

Certificate from the Supervisor

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

Signature of the Supervisor:.....

Name & Designation: **Dr. Shamsuzzaman**
Professor of Chemistry

Department: **CHEMISTRY**

Chairman
Department of Chemistry
A.M.U., Aligarh

(Signature of the Chairman of the Department with seal)

COURSE/ COMPREHENSIVE EXAMINATION/ PRE-SUBMISSION SEMINAR COMPLETION CERTIFICATE

This is to certify that Mr. **Ashraf Ahmed Hasan Mashrai**, Department of **chemistry** has satisfactory completed the course work/comprehensive examination and pre-submission seminar requirement which is part of his Ph.D programme.

Date: 31.3.2014

(Signature of the Chairman of the Department)

Chairman
Department of Chemistry
A.M.U., Aligarh

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Candidate's Name: ASHRAF AHMED HASAN MASHRAI

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(Signature of the Candidate)

Dedicated to

My family,
My country (Yemen) and
My supervisor professor Shamsuzzaman

Acknowledgments

All praise and thanks belong to ALLAH (SWT) Alone, The Exalted, Almighty, All-Glorious, as befits His glory and the greatness of His power. I thank Him for the blessing which He has bestowed upon me, and for honouring me with His aid to accomplish this research work.

I wish to express the deep sense of gratitude to my research advisor, **Prof. Shamsuzzaman**, whose encouragement and guidance were a source of great inspiration to me. It was his constant support, supervision, advice and kind co-operation which helped me to build an optimistic attitude towards my research work. His invaluable helpful suggestions, enlightening explanations and assistance were indispensable throughout the work.

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Of course, I would not be where I am today without the great love and support from my family. My parents have been encouraging from the beginning, allowing me to choose my own path. My pursuit of higher education has been a long journey, and for the opportunities afforded to me by my Father (**Ahmed Hasan Mashrai**) and Mother (**Salma Ali Galad**), I am forever grateful. I am also very grateful to my brothers (**Abdullah**, **Mohammed**, **Mustafa**, **Anwar**, **Jamel**, **Majed** and **Khalid**) and sisters (**Eman and Badreah**) for their support throughout my research and for their help during the challenging time.

I wish to convey my heartiest thanks to **Mr. Fareed Ahmad Mugawwar, the Minister of Agriculture and Waters, Yemen, and Sheikh Ahmad Saleh Al-Eisi** for their gracious support.

In particular huge thanks must go to my lab colleagues **Hena Khanam, Ayaz M. Dar, Mohd Asif and Abad Ali** for having shared many experiences and thoughts with me throughout the research period.

I wish to convey my thanks to my friends who were always with me throughout this tenure, helping me in all situations especially **Saleem Garandal, Fadhl Fushoosh,**

Nabeel Agrah, Muhsen Al-Hamed, Abdul-Rahim Mugawwar, Mohammad Nahari, Dr. Ibrahim Hydarh, Dr. Sulaiman Al-Safi and **Dr. Niyazi Al-Areqi**. Their constant support, trust and moral boost together with enormous motivation proved to be priceless.

Thanks are also due to **Nayeem Ahmed**, **Tawseef Ahmad**, **Himanshu Gupta**, **Ali Mohammed** and **Md. Fahimul Hassan**, who ungrudgingly provided me with immense help and support.

I would like to acknowledge University Grants Commission (UGC) New Delhi India, for providing me JRF fellowship during this tenure.

I also acknowledge Instrumentation Centre Department of Chemistry, USIF Aligarh Muslim University, Aligarh for providing spectral data.

"The mind is not a vessel to be filled but a fire to be kindled."

Plutarch

(Ashraf Ahmed Hasan Mashrai)

CONTENTS

Summary

In the field of medicinal chemistry, steroids have been one of the most attractive areas for investigation due to their biological activities. Bioorganic chemists have reported a number of modifications in natural steroids resulting in the promotion of their activities. Even a small change in steroid moiety can elicit an extensive biological response. All these facts have attracted researcher to explore these after modifying them suitably to induce various pharmacological properties. This is still to be a fascinating field worldwide. Our laboratory is concerned mainly with the synthesis of steroidal compounds and their identification by chemical and spectral studies, a number of reports dealing with the preparation of modified steroids have appeared from our laboratory. The work embodied in this thesis describes the synthesis of modified steroids and their biological behavior. The identification of newly synthesized compounds has been ascertained by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. The whole work is divided into five chapters namely,

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Chapter-2: Steroidal pyranones

Chapter-3: Steroidal pyrazolones

Chapter-4: Steroidal thiazolidinones

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Steroidal pyrazolines

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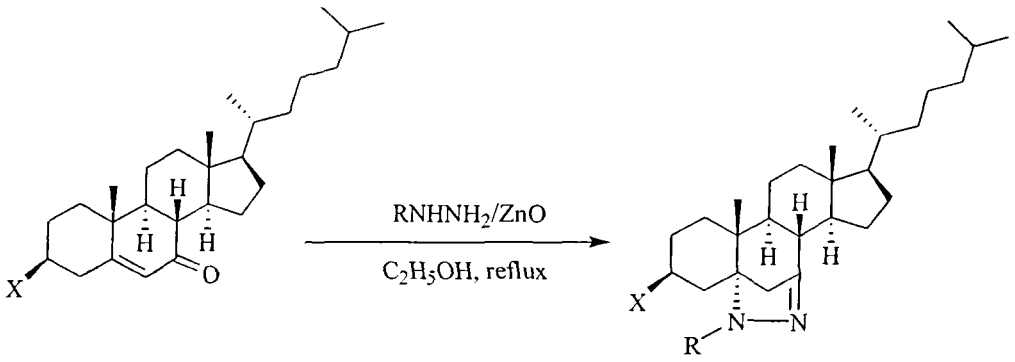

(I-III)		(IV-IX)				
X		**X**	**R**		**R**	
H	**(I)**	H	H	**(IV)**	Ph	**(VII)**
OAc	**(II)**	OAc	H	**(V)**	Ph	**(VIII)**
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Steroidal pyranones

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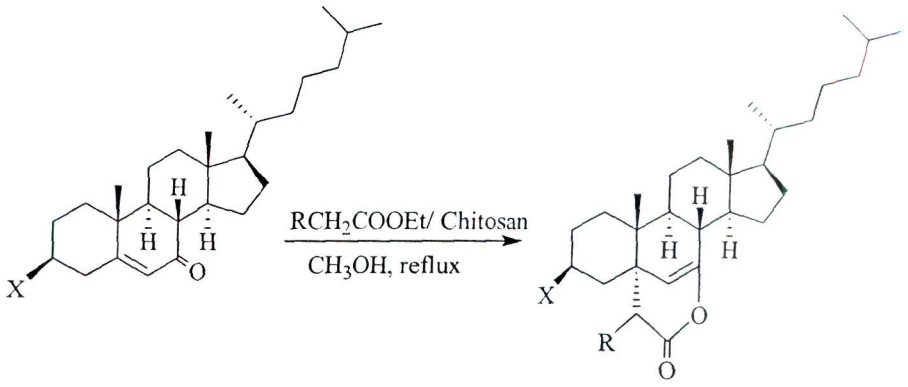

(I-III) **(X-XV)**

X		X	R		R	
H	(**I**)	H	Cl	(**X**)	CH_3CO	(**XIII**)
OAc	(**II**)	OAc	Cl	(**XI**)	CH_3CO	(**XIV**)
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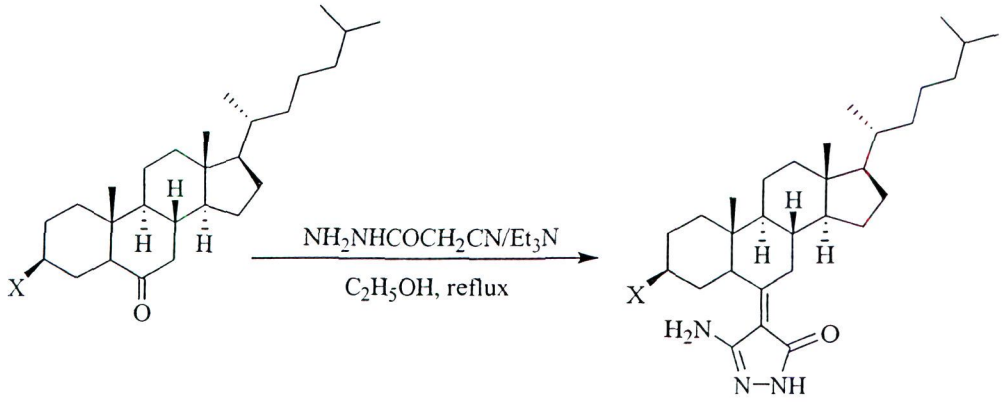

(XVI-XVIII)		(XIX-XXI)	
X		**X**	
H	(**XVI**)	H	(**XIX**)
OAc	(**XVII**)	OAc	(**XX**)
Cl	(**XVIII**)	Cl	(**XXI**)

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CHAPTER-4

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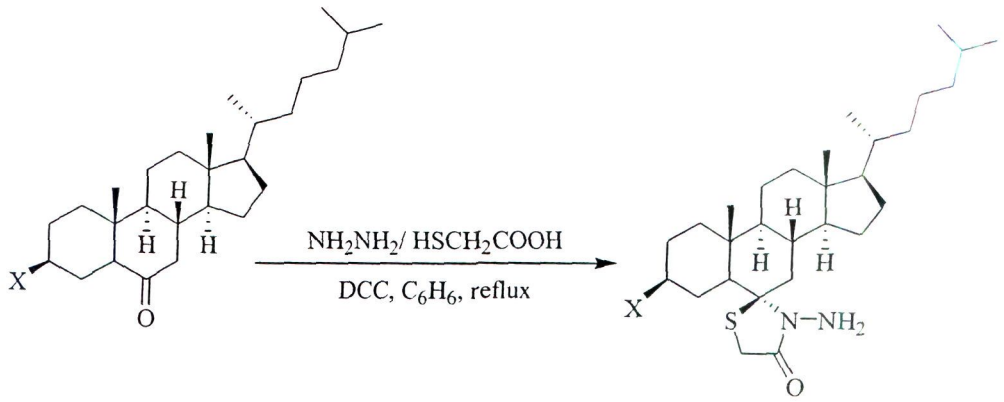


(XVI-XVIII)		(XXII-XXIV)	
X		X	
H	(XVI)	H	(XXII)
OAc	(XVII)	OAc	(XXIII)
Cl	(XVIII)	Cl	(XXIV)

CHAPTER-5

Biological evaluation of newly synthesized compounds

The global production of new drugs has increased in the last decades, and although many of the products have been beneficial for mankind, many of them are also toxic and can accumulate within organisms. In the global drug market, steroid drugs rank second only after antibiotics. Search of novel drugs is field of current and growing interest and many compounds have been synthesized to this aim. One possibility to achieve this goal is the combination of a steroid molecule with structural elements possessing appropriate biological activities. The advantage of employing hydrophobic steroid units is their ability to interact with cell membranes and thus pave the way for biological activity of such hybrid molecules. To gain insight on how modifications on ring-B in steroids nucleus can affect the biological activity, different steroidal derivatives with a variety of functionalities attached to steroidal skeleton have been synthesized and evaluated *in silico* and *in vitro* for their biological activity. In the *in slico* studies, we studied the physicochemical parameters "Rule of Five" of the synthesized compounds and we calculated the bioactivity score for six criteria, GPCR ligand activity, ion channel modulation, kinase inhibition activity, protease inhibitor, enzyme inhibitor and nuclear receptor ligand activity. The cytotoxic potential of the synthesized compounds were studied against panel of selective human cancer cells by MTT assay. The panel of cancer cells encompassed **HepG2** (hepatocellular carcinoma), **A549** (lung adenocarcinoma epithelium), **SW480** (colon adenocarcinoma), **HeLa** (cervical carcinoma) and **HL-60** from (promyelocytic leukaemia). Doxorubicin and Cytarabine were used as cytotoxic drugs of reference. The compounds were also tested against one normal cell line **PBMC** (Blood peripheral mononuclear cell isolation). The newly synthesized compounds were screened against a variety of bacterial strains (*Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae*) by disk diffusion method. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 36 °C. The minimum inhibitory concentration (MIC) of all the compounds was determined. Ciprofloxacin was used as positive control, whereas DMSO poured disk was used as negative control and then minimum inhibitory

concentration (MIC) was evaluated by the macro-dilution test. The *in vitro* antifungal activities were carried out using (*Candida albicans, Aspergillus fumigatus, Trichophyton mentagrophytes* and *Penicillium marneffei*) by agar diffusion method. The minimum inhibitory concentration (MIC) and Inhibition zones were determined as in antibacterial activity. The activity of the synthesized compounds was compared with Fluconazole as positive control and DMSO as negative control. The synthesized compounds were also tested for antioxidant activities by 1,1diphenylpicrylhydrazyl (DPPH) method. GOLD (Genetic Optimization for Ligand Docking) 5.0 was used for docking of compounds **(XIX- XXIV)** against S12 and CYP 51 proteins.

Introduction

Steroids have been a prime focus of research due to their fascinating structural framework and their excellent ability to penetrate cell membranes and bind to the nuclear and membrane receptors. Even a small change in steroid moiety can elicit an extensive biological response. Steroids represent constituents of biomembranes and hormones, fulfil protective functions, stimulate plant growth, etc. Many representatives of this group are widely used in medicine as essentials of anti-inflammatory, anabolic and contraceptive drugs. All these facts have attracted medicinal chemists and biochemists to explore these after modifying them suitably to induce various pharmacological properties. It is therefore not surprising that steroids considerably interest not only chemists, but also pharmacologists and physicians.

Steroids research in the mid-to late 20th century progressed to biomimetic chemistry and tracer work medicinal and pharmacological chemistry as well. During the early and middle years of steroid chemistry, synthetic efforts focused primarily upon the ring system and some of the more simple functional side chains. These studies were directed primarily towards the development of synthetic methods for the construction and modification of the cyclic skeleton and were due, of course, to the demand for potent pharmaceutical agents. In addition, progress in synthetic methods and separation and identification techniques prompted more detailed studies of these derivatives. Within the last decade intensive research on modified steroids has yielded many imaginative syntheses of general interest and has contributed much to the development of novel biologically potent compounds.

Our laboratory is mainly concerned with the synthesis of steroid derivatives and their identification by spectral and analytical studies. The aim of the present work then is to survey the synthesis of modified steroids as well as some relevant chemistry involving transformations of the ring A and B for the preparation of compounds of biological importance. It also attempts to provide steroid chemists pursuing new syntheses of steroids or compounds with similar structures. This doctoral thesis comprises the design and synthesis of modified steroids and their biological screening.

CHAPTER-1

Steroidal Pyrazolines

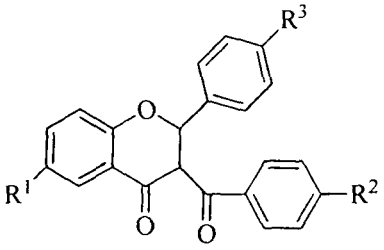
Theoretical

The pyrazoline ring (**1**) system consists of a monounsaturated five membered ring containing two adjacent nitrogen atoms.

(1)

Over the years, the synthesis of pyrazolines has received considerable attention because of their noteworthy applications. Certain compounds containing the pyrazoline moiety were synthesized to have an important therapeutic potential, mainly as anti-inflammatory, antidepressant, antipyretic, antibacterial, antifungal and antitumor agents. That's why preparation of pyrazolines itself shows remarkable interest and is thus highly attractive area of synthetic organic chemistry.

Assad[1] synthesized pyrazolines and pyrazoles from *p*-hydroxyacetophenone using NH_2NH_2 in acetic acid and evaluated their antimicrobial properties. Chincholkar and Jamode[2] synthesized 4-aroyl-subsituted pyrazolines (**3**) and pyrazoles (**4**) [R^1 = H, Me, R^2 = H, MeO, R^3 = H, MeO, 3,4-methylenedioxyl] by cyclocondensation of 3-aroylflavanones (**3**) with $PhNHNH_2$.



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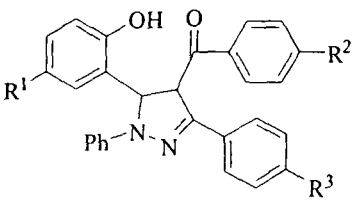

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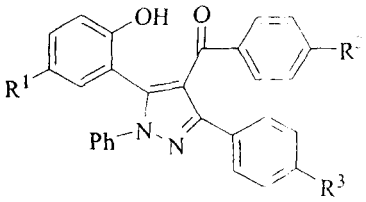
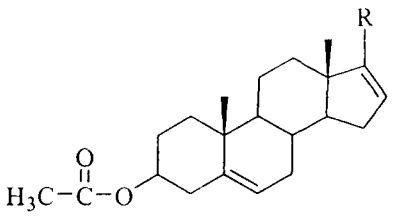


(4)

Green *et al.*[3] synthesized steroidal [16α,17 α-*d*]-2′-pyrazolines (**6**) and [16,17-*d*] pyrazoles (**7**). 3β-Acetoxyandrostadienes (**5**) [R = MeCO] with PhCCl: NNHPh/ Et_3N gave steroidal pyrazolines (**6**) and similar cycloaddition reactions of compound **5** [R = AcO] provided androstenopyrazoles (**7**) [R = Ph, R^1 = Ph, 4-ClC_6H_4].



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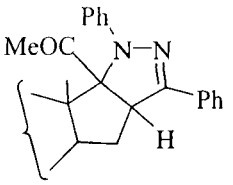

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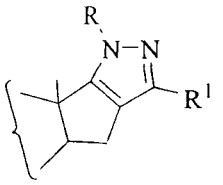
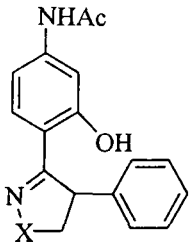


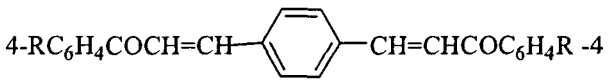
(7)

2′-Hydroxy-4′-acetamidochalcones on reaction with NH_2OH in ethanol provided isoxazoline (**8**) [X=O] while with NH_2NH_2 and $PhNHNH_2$, respective pyrazolines (**8**) [X = NH and NPh] were obtained.[4]



(8)

Doyl *et al.*[5] carried out the dipolar addition of diazocarbonyl compounds to α,β-unsaturated esters and nitriles in the presence of pyridine, which resulted in the formation of 2-pyrazolines. Halo and hydroxyl aryl pyrazolines were synthesized by Latif *et al.*[6] Cycloaddition of CH_2N_2 to $RC_6H_4SO_2$ CH: CH [R = H, 4-Br, 4-Cl, 2-Me, 4-Me] in the presence of Et_3N gave 3-aryl sulphonyl-2-pyrazoline.[7] The dichalcones (**9**) [R = H, F, Cl, Br, MeO] were cyclized with R^1NHNH_2 [R^1 = H, Me, Ph] to give the dipyrazolines (**10**).[8]



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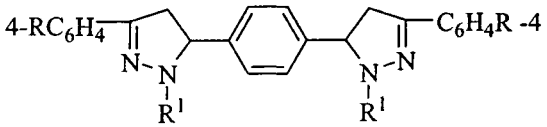
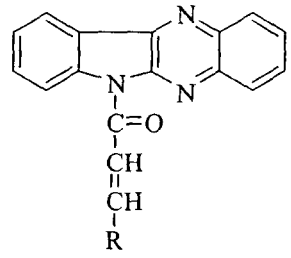


(10)

Cyclization of ketones (**11**) with NH_2NH_2, $PhNHNH_2$ and NH_2OH produced pyrazolines and isoxazolines (**12**)[9] [R=Ph, 4-$MeOC_6H_4$, 4-ClC_6H_4, 4-$O_2NC_6H_4$, PhCH:CH, X = NAc, NPh, O] respectively. Some 3-aroyl-4-aryl-2-pyrazol nes[10] were prepared by condensation of α,β-unsaturated ketones with diazomethane in Et_3N.



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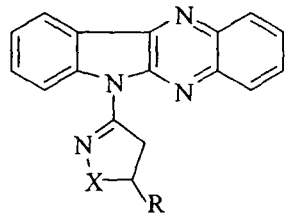
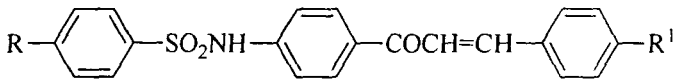


(12)

Kumar and Hussain[11] prepared N-[4-(acetyl/phenyl-5-aryl pyrazoline-3-yl) phenyl] aryl sulphonamides (**14**) [R = H, Me, R^1 = H, Cl, OMe, Me, NO_2, R^2 = Ac, Ph] by cyclization of compound (**13**) with NH_2NH_2 and $PhNHNH_2$.



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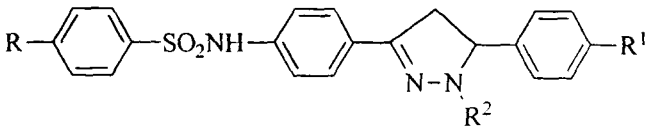
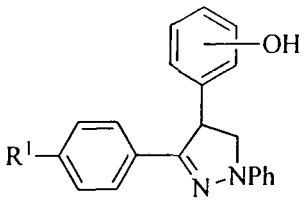
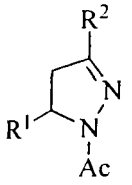


(14)

Some 1,3,5-triphenyl-3-pyrazoline derivatives (**15**)[12] [R^1 = H, OMe, Cl] were synthesized by heating 3- and 4-hydroxy chalcone derivatives with $PhNHNH_2$ using

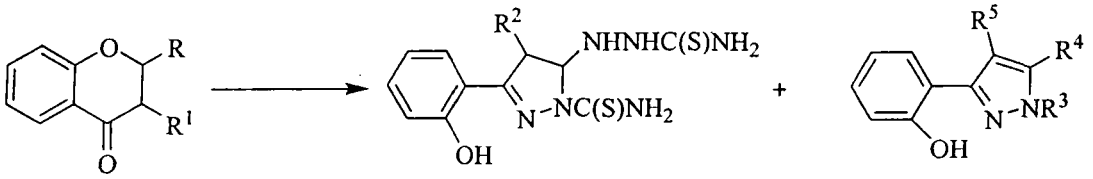
acetic acid as catalyst. The synthesized compounds were found to possess antifungal activity. Mishriky *et al.*[13] reported the synthesis of 1-acetyl-3,5-diaryl-Δ^2-pyrazolines (**16**) [R^1 = Ph, anisyl, $HO(MeO)C_6H_3$, R^2 = $Cl_2C_6H_3$, FC_6H_4] by reacting chalcones with NH_2NH_2 in AcOH. The formation of aflatoxin by *Aspergillus flavus* was inhibited by compound **16**.





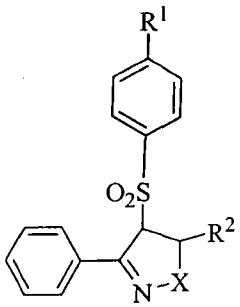
(15) **(16)**

Maib and Jerzmanowaska[14] carried out the reaction of chromones (**17**) [R = R^1 = H, Me] with $H_2NNHCSNH_2$ to give pyrazolines (**18**) [R^2 = H, Me] and pyrazoles (**19**) [R^3 = H, $CSNH_2$, R^4=R^5 = H, Me].



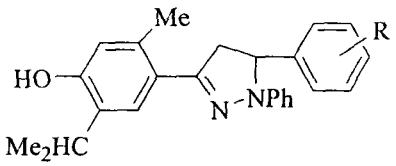
(17) **(18)** **(19)**

Shingare and Siddiqui[15] reported the formation of arylsulfonyl pyrazolines and isoxazolines (**20**) [X = NH, NPh, O, R = H, Me, R^1 = H, Me, Cl, R^2 = substituted Ph, heteroaryl] by the cyclocondensation of chalcones with NH_2NH_2, $PhNHNH_2$ or NH_2OH.

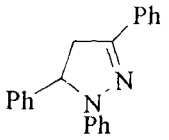


(20)

Roda *et al.*[16] prepared 5-aryl-1-phenyl-3-(3-isopropyl-4-hydroxy-6-methylphenyl)-2-pyrazolines (**21**) [R = H, 2-, 3-, 4-OH, 2- and 4-Cl, 3- and 4-NO_2, 4-OMe, 4-NMe_2, etc.] by cyclocondensation of the chalcones with $PhNHNH_2$. Kleefeld and Dutzmann[17] synthesized 1,3,5-triphenyl-2-pyrazoline (**22**) by reacting 1-phenyl-3-(4-hydroxy phenyl)-2-propen-1-one and $PhNHNH_2$ in AcOH. They were used for the control of *Erysiphe graminis*.

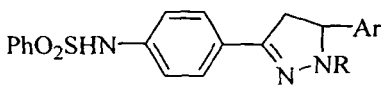


(**21**)

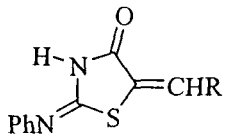


(**22**)

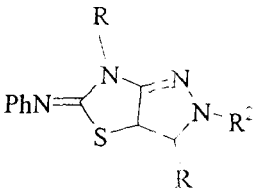
Upadhyay *et al.*[18] cyclized 4-$PhSO_2NHC_6H_4COCH$: CHAr [Ar = Ph, 2-$O_2NC_6H_4$, 3-ClC_6H_4, 2-hydroxy-1-naphthyl, etc.] with NH_2NH_2 / AcOH or $PhNHNH_2$ to give pyrazolines (**23**) [R = Ac, Ph]. Singh[19] reported the condensation reaction of substituted thiazolidinones with PhCHO or *p*-anisaldehyde in the presence of NaOAc in AcOH to give phenyliminobenzylidene thiazolidinones (**24**) [R = Ph, R^1 = H, Ph, R = 4-$MeOC_6H_4$, R^1 = Ph], which on cyclization with hydrazines gave thiazolidinopyrazolines (**25**) [R = Ph, R^1 = H, Ph, R = Ph, R^1 = Ph, R^2 = H, Ph, R = 4-$MeOC_6H_4$, R^1 = Ph, R^2 = H].



(**23**)

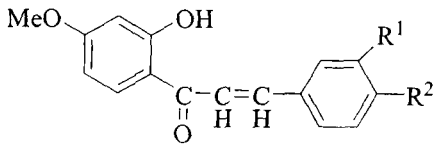


(**24**)

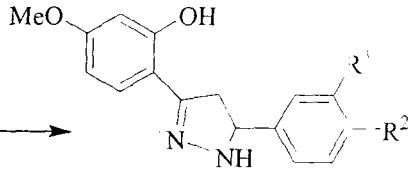


(**25**)

Sonare and Doshi[20] carried out the condensation of 2′-hydroxy-4′-methoxy chalcones (**26**) [R^1 = OMe, R^2 = H, R^1 = R^2 = H, R^1=R^2 = OCH_2O] with NH_2NH_2 in ethanol to give 1*H*-3-(2′-hydroxy-4′-methoxy)-5-substituted phenyl-2-pyrazolines (**27**).

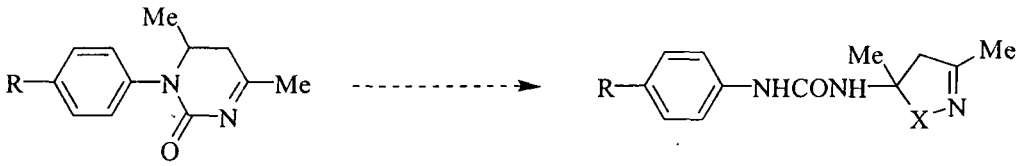


(**26**)



(**27**)

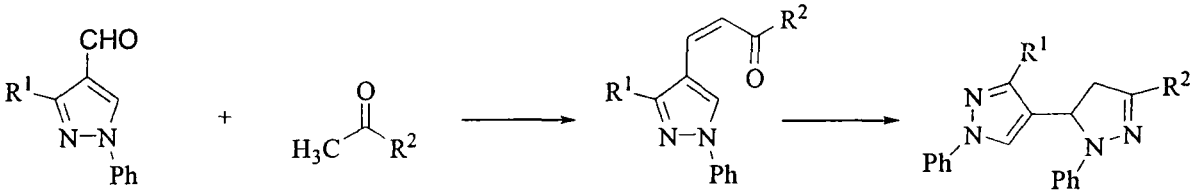
1-Aryl-4,6-dimethyl pyrimidin-2 (1*H*)-ones (**28**) [R = H, Me, OMe] with NH_2OH and $AcNHNH_2$ led to isoxazolines and pyrazolines (**29**).[21] [same R, X = O, NAc] respectively. Compounds **30** and **31** were prepared by the reaction[22] of araldehyde hydrazones and araldoximes with bifunctional olefins in the presence of chloramine-T.



(28) (29)

(30) (31)

Bratenko *et al.*[23] synthesized 1-aryl (heteryl)-3-[3-aryl (heteryl)-4 pyrazolyl] propenones (**34**) [R^1 = Ph, R^2 = Ph, 4-FC_6H_4, 4-ClC_6H_4, 4-BrC_6H_4, 4-$MeOC_6H_4$] by condensation of 4-aryl (heteryl)-4-formylpyrazoles (**32**) [R^1 = Ph, 2-thienyl, 5-methyl-2-furyl, 3-pyridyl] with methyl aryl (heteryl) ketones (**33**) [R^2 = Ph, 4-FC_6H_4, 4-ClC_6H_4, 4-BrC_6H_4, 4-EtC_6H_4, 4-$MeOC_6H_4$]. The compound **34** on reaction with phenylhydrazine yielded 1-phenyl-3-aryl (heteryl)-5-(4-pyrazolyl)-2-pyrazolines (**35**) [R^1 = Ph, R^2 = Ph, 4-FC_6H_4, 4-ClC_6H_4, 4-BrC_6H_4, 4-EtC_6H_4, 4-$MeOC_6H_4$].

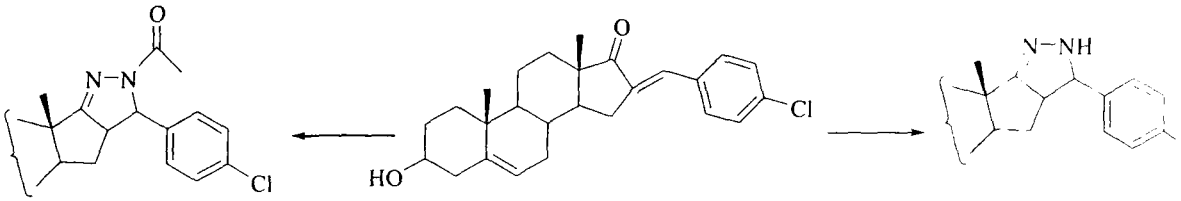


(32) (33) (34) (35)

Pimenov *et al.*[24] carried out the reaction of ketones (**36**) [n = 0, 1, 0 R = H, H. NO_2] with 99% hydrazine hydrate under acidic conditions (acetic acid, sulfuric acid) which gave cyclic product 5-(1-adamantyl)-3-phenylpyrazoline (**37**).

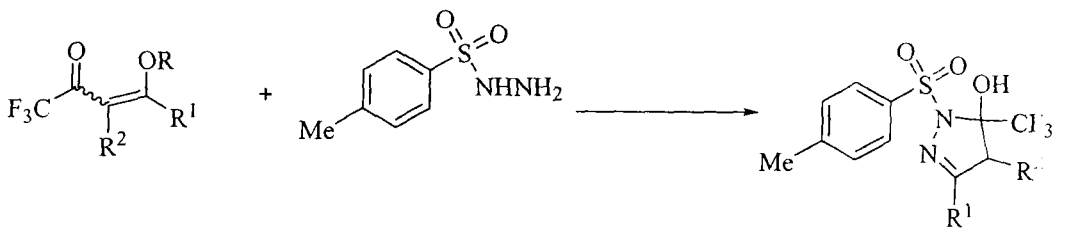
(**36**) (**37**)

Condensation of 3β-hydroxy-16-[(4-chlorophenyl) methylene]-androst-5-en-17-one (**38**) with hydrazine hydrate in acetic acid afforded 1'-acetyl-1'-*H*-5'-(4-chlorophenyl) androst-5-en-[17,16- *c*]-pyrazoline-3β-ol (**39**). 1'-*H*-5'-(4-chlorophenyl)-androst-5-en-[17,16- *c*]-pyrazoline-3β-ol (**40**)[25] was also prepared by refluxing the compound **38** and hydrazine hydrate in dioxane for 5 h.



(**39**) (**38**) (**40**)

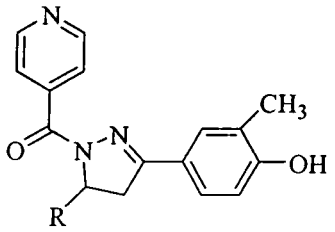
Bonacorso *et al.*[26] reported the regiospecific synthesis of a novel series of 4-phenyl- and 3-alkyl(aryl)-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-1-tosylpyrazoles (pyrazolinyl *p*-tolyl sulfones) (**43**). The 1-*p*-tosyl-2-pyrazolines were obtained from the cyclocondensation reaction of 3-phenyl- and 4-alkyl(aryl)-1,1,1-trifluoro-4-alkoxy-3-alken-2-ones (**41**), [where alkyl are H, Me and aryl are -C_6H_5, 4-$CH_3C_6H_4$, 4-$OCH_3C_6H_4$, 4-FC_6H_4, 4-ClC_6H_4, 4-BrC_6H_4] with *p*-tosylhydrazine in a yield of 58 to 92% when toluene was employed as solvent. Their antimicrobial activities were also studied.



(**41**) (**42**) (**43**)

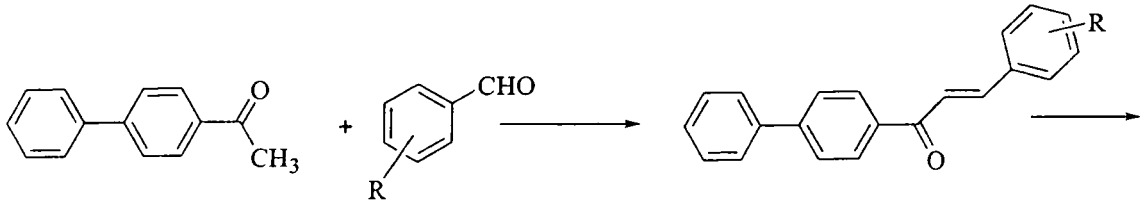
R	Et	Me	Me	Me	Me	Me	Me	Me	Me
R^1	H	Me	Ph	4-MePh	4-MeOPh	4-BrPh	4-ClPh	4-FPh	H
R^2	H	H	H	H	H	H	H	H	Ph

A series of N^1-nicotinoyl-3-(4′-hydroxy-3′-methyl phenyl)-5-(substituted phenyl)-2-pyrazolines (**44**)[27] were synthesized by the reaction between isoniazid (INH) and chalcones in the presence of acetic acid. [R = 4-methoxy phenyl, 4-chloro phenyl, 4-dimethylamino phenyl, Phenyl, 3,4-dimethoxy phenyl, 2,3,4-trimethoxy phenyl, furyl, 4-fluoro phenyl, 2-chloro phenyl, 2,6-dichloro phenyl, 3-nitro phenyl].

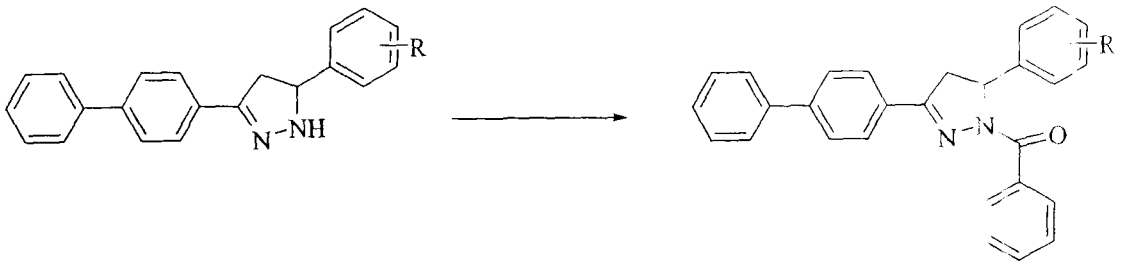


(44)

Amir *et al.*[28] synthesized a series of 3-(4-biphenyl)-5-substituted phenyl-2-pyrazolines (**49**) [R = H, 2-Cl, 4-Cl, 4-$N(CH_3)_2$, 4-CH_3, 4-OCH_3, 3,4-$(OCH_3)_2$, 2,4,6-$(OCH_3)_3$] and 1-benzoyl-3-(4-biphenyl)-5-substituted phenyl-2-pyrazolines (**48**) [R, same as above] by condensation of chalcones with hydrazine hydrate. 3-Aryl-1-(4-biphenyl) propen-1-ones (**47**) [R, same as above] were synthesized by treating 4-acetyl biphenyl with aromatic aldehydes in the presence of methanol-dioxane and potassium hydroxide solution. These upon treatment with hydrazine hydrate and few drops of concentrated HCl, gave 3-(4-biphenyl)-5-substituted phenyl-2-pyrazolines (**48**). Reaction of compounds **48** with benzoyl chloride in presence of pyrimidine gave 1-benzoyl-3-(4-biphenyl)-5-substituted phenyl-2-pyrazolines (**49**).

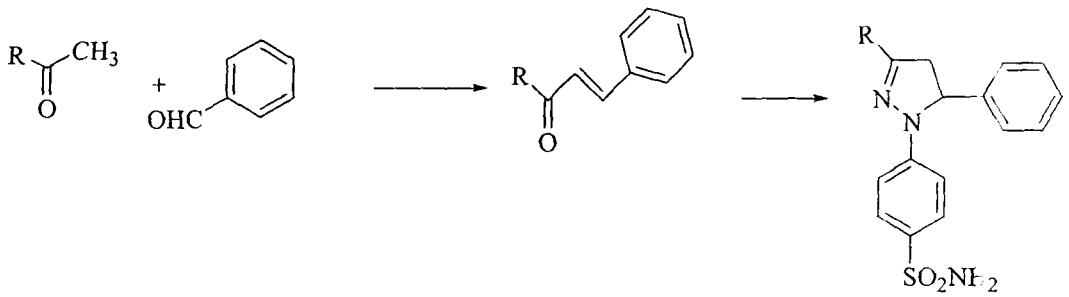


(45) **(46)** **(47)**

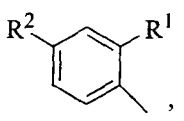
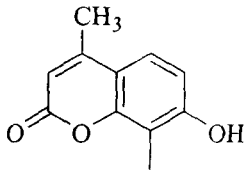


(48) (49)

Rathish *et al.*[29] carried out the reaction of chalcones (**52**) with 4-hydrazinon benzenesulfonamide hydrochloride and obtained 1,3,5-trisubstituted pyrazolines bearing benzene sulfonamides (**53**). The intermediate chalcones (**52**) were prepared by base catalyzed Claisen-Schmidt condensation of the aromatic ketones (**51**) with different aromatic aldehydes.



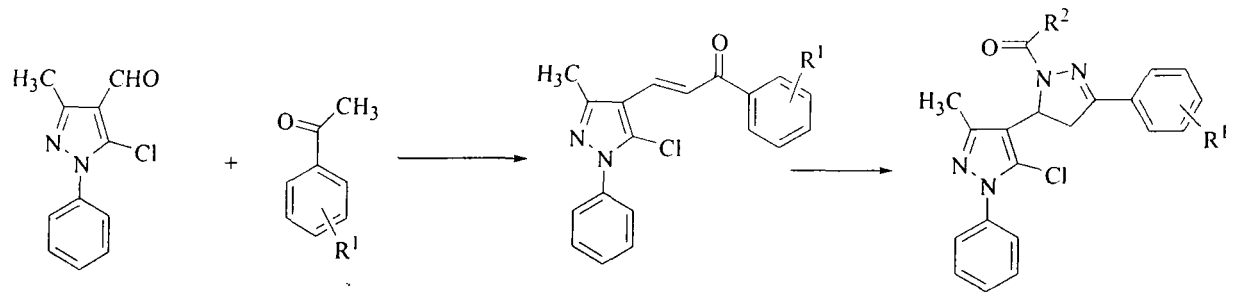
(50) (51) (52) (53)

R =  , 

50a, **52a-g**, **53a-g**; R^1 = OH, R^2 = OCH_3, **50d**, **52r**, **52s**, **53r**, **53s**
50b, **52h-o**, **53h-o**; R^1 = R^2 = OCH_3
50c, **52p**, **52q**, **53p**, **53q**; R^1 = OH, R^2 = H

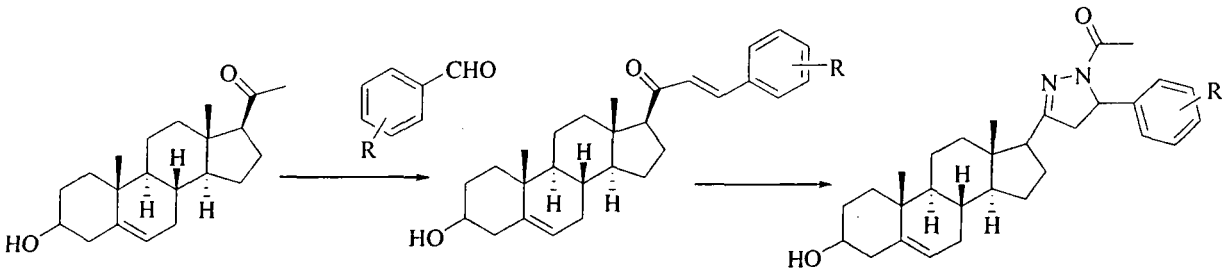
A series of 1-acetyl/propyl-3-aryl-5-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2-pyrazolines (**57**) [R^1 = H, 4-CH_3, 4-OCH_3, 3-NO_2, 4-Cl, 4-Br, 4-NO_2, R^2 = CH_3, CH_3CH_2] were synthesized by Girisha *et al.*[30] in one step by condensing suitably substituted propenones, $NH_2NH_2.H_2O$ and acetic/propionic acid. The 3-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(aryl)-2-propen-1-one (**56**) [R^1 same as above] was prepared by the reaction of aldehyde (**54**) with appropriately substituted

acetophenones (**55**) [R^1 same as above]. These compounds were also screened for analgesic and anti-inflammatory activity.



(54) **(55)** **(56)** **(57)**

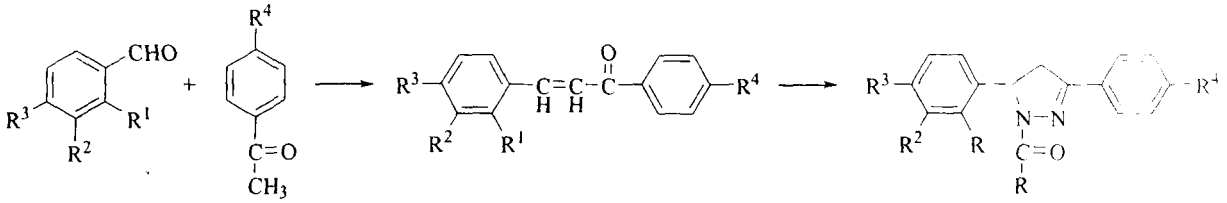
Banday *et al.*[31] synthesized 17-pyrazolinyl derivatives of pregnenolone (**60**) [R = C_6H_5, 3-FC_6H_5, 4-FC_6H_5, 4-$CH_3C_6H_5$, 2-$CH_3C_6H_5$, 3-$CH_3C_6H_5$, C_4H_3O, 4-$OMeC_6H_5$, 2-$OMeC_6H_5$, 2-ClC_6H_5] from the chalcone (**59**) in the presence of $NH_2NH_2.H_2O$ and AcOH. Compound **59** was prepared by condensation of pregnenolone (**58**) with various aromatic aldehydes.



(58) **(59)** **(60)**

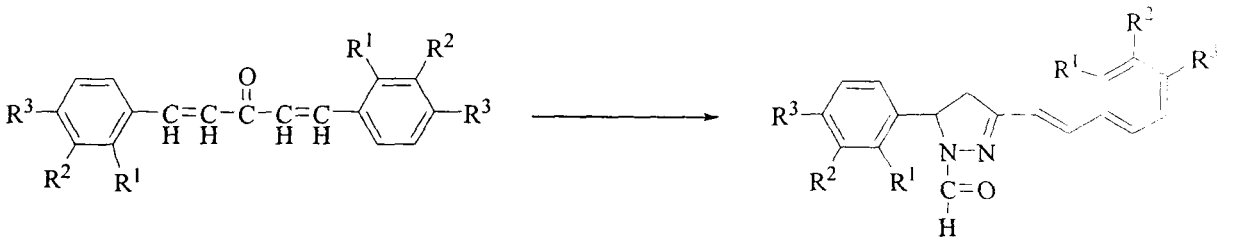
Pant *et al.*[32] carried out the synthesis of 3,5-diaryl-N-substituted pyrazolines (**64**) from α,β-unsaturated ketones (**63**) and hydrazine hydrate with acetic/formic acid in ethanol/DMSO. Compounds (**63**) were prepared by condensation reaction of various aromatic aldehydes and ketones. [**63a**, **63b**: R^1= R^2= R^3= H, R= CH_3; **63h**, **63i**: R = R^1= R^2= H, R^3= R^4 = Br; **62a**, **63b**: R = R^1= R^2 = R^3= R^4 = H; **62i**, **63j**: R= R^2= R^3= H, R^1= Cl, R^4 = Br; **62b**, **63c**: R = R^1= R^2 = R^3= H, R^4 = NO_2; **62j**, **63k**: R = R^1= R^2= H, R^3= Br, R^4 = NO_2; **62c**, **63d**: R = R^1= R^2=R^4 = H, R^3= Cl; **62k**, **63l**: R = R^1= R^2= H, R^3= Cl, R^4 = NO_2; **62d**, **63e**: R = R^1= R^3= R^4 = H, R^2= NO_2; **62l**, **63m**: R

= R^1= R^3= H, R^2= NO_2, R^4 = Br; **62e**, **63f**: R = R^1= R^3 = H, R^2= R^4= NO_2; **62m**, **63n**: R = R^1= R^2= R^3= H, R^4 = Cl; **62f**, **63g**: R = R^1= R^2=R^3= H, R^4 = Br; **62n**, **63o**: R = R^1= R^2 = R^3 = H, R^4 = OH; **62g**, **63h**: R = R^1= R^2=R^4 = H, R^3= Br; **62o**, **63p**: R = R^2= R^3=H, R^1= Cl, R^4= OH].



(61) **(62)** **(63)** **(64)**

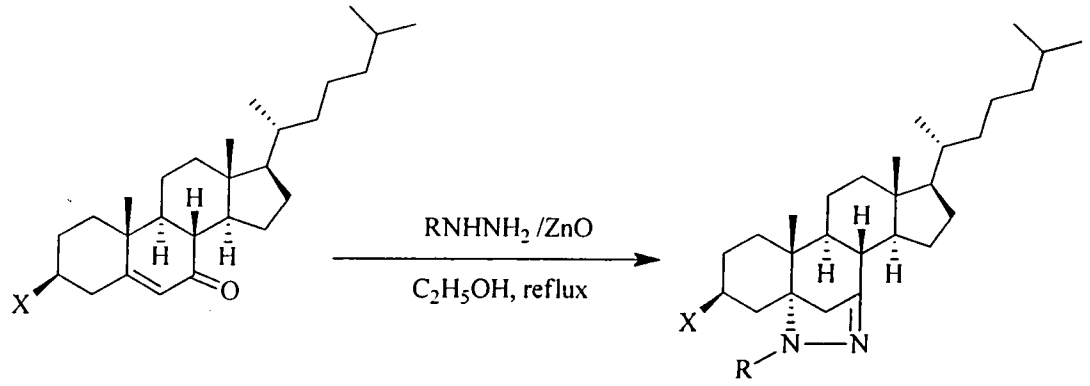
Singh *et al.*[33] reported the synthesis of pyrazoline derivatives (**66**) [R^1 = R^2 = R^3= H, R^1 = R^3 = H, R^2 = NO_2, R^2 = R^3 = H, R^1= Cl, R^1= R^2 = H, R^3 = Cl, R^1 = R^2 = H, R^3= Br] from dibenzalacetones (**65**) [R^1, R^2, R^3 same as above], formic acid and hydrazine hydrate in ethanol. They also studied photophysical properties of these compounds.



(65) **(66)**

Discussion

Pyrazoline is an important nitrogenous five-membered heterocyclic component of the drugs. Literature survey revealed that numerous pyrazoline derivatives have found their clinical application. Several analogies of pyrazolidin-3,5-diones, pyrazolin-3-ones and pyrazolin-5-ones are also available as drugs; examples are felcobuzone, mefobutazone, morazone, famprofazone and ramifenazone[34]. Besides these, many pyrazoline derivatives have been found to possess a broad spectrum of biological activities such as antitumor, [35] anti-inflammatory,[36] MAO-B inhibitors,[37]agonist of cannabinoid receptors[38] and antioxidant.[39] Out of these biological activities of pyrazolines and their derivatives, antimicrobial and anticancer activities are of particular interest. Many pyrazoline derivatives are also reported in the literature as having potent antifungal activity,[40] which were assayed against *Cephalosporium acremonium*, *Helminthosporium oryzae* and *Acheya orion* and were found extensively active. In view of these reports and in continuation of our previous work[41] in steroidal chemistry, we have synthesized some new steroids containing pyrazoline ring fused with steroidal ring-B by using biosynthesized ZnO nanoparticles as a catalyst. The substrates selected for initial studies include cholest-5-en-7-one (**67**), 3β-acetoxycholest-5-en-7-one (**68**) and 3β-chlorocholest-5-en-7-one (**69**)[42]. The attractive features of this protocol are simple reaction procedure, short reaction time and easy products. The structures of newly synthesized compounds have been assigned on the basis of elemental analysis and spectral (IR, ^{1}H NMR, ^{13}C NMR, MS) studies. To the best of our knowledge, this is the first report about the synthesis of steroidal pyrazolines using nano metal oxide as a catalyst.



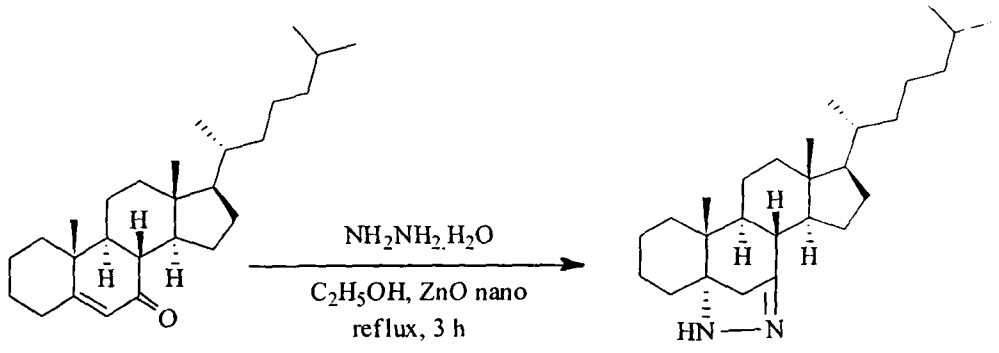
(67-69)	
X	
H	**(67)**
OAc	**(68)**
Cl	**(69)**

(70-75)				
X	**R**		**R**	
H	H	**(70)**	Ph	**(73)**
OAc	H	**(71)**	Ph	**(74)**
Cl	H	**(72)**	Ph	**(75)**

Biological synthesis of ZnO nanoparticles using C. albicans and studying their Catalytic performance in the synthesis of steroidal pyrazolines. Shamsuzzaman, **Ashraf Mashrai**, Hena Khanam, Rezq N. Aljawfi
Arabian J. Chem. **2013** (in press);http://dx.doi.org/10.1016/j.arabjc.2013.05.004

Reaction of cholest-5-en-7-one (67) with hydrazine hydrate: 5α-Cholestano-[5, 7- c d]-pyrazoline (70):

Steroidal α,β-unsaturated ketone **67** in ethanol was allowed to react with hydrazine hydrate under reflux condition in the presence of ZnO nanoparticles for 3 h, after usual work up and recrystallization a single product **70**, m.p. 134-136 °C, was obtained.



(67) **(70)**

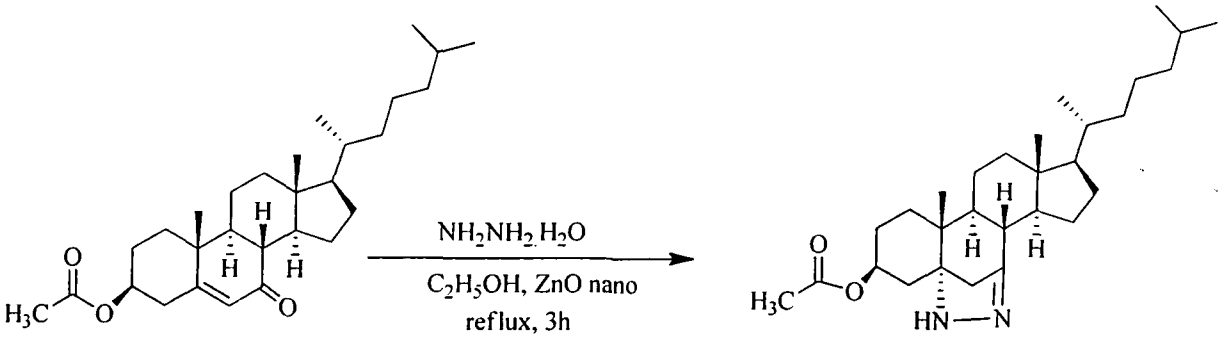
Characterization of compound 70 as 5α-cholestano-[5, 7- c d]-pyrazoline:

The elemental analysis of compound **70** corresponded to the molecular formula $C_{27}H_{46}N_2$.The IR data provided evidence for the formation of the expected compound. The compound showed intense band in the region of 3267 cm^{-1} due to N-H stretching vibration. In addition, other important absorption band at 1657 cm^{-1} was attributed to C=N stretching. Further evidence for the formation of compound **70** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited one-proton singlet at δ 5.2 for NH (exchangeable with D_2O). Angular and side-chain methyl protons were observed at δ 1.19 (C10-CH_3), 0.75 (C13-CH_3), 0.91 and 0.85 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 155 for *C*=N and 45 for *C*-N, in addition to the signals of cholestane series. The mass spectrum was also in good agreement with its molecular formula which exhibited strong molecular ion peak at *m/z* 398.

On account of the above descriptive discussion, the compound **70** can be suitably characterized as *5α-cholestano-[5, 7- c d]-pyrazoline.*

cholestano-[5, 7- c d]-pyrazoline (71):

Steroidal α,β-unsaturated ketone **68** in ethanol was allowed to react with hydrazine hydrate under reflux condition in the presence of ZnO nanoparticles for 3 h, after usual work up and recrystallization a single product **71**, m.p. 137-139 °C, was obtained.



(68) (71)

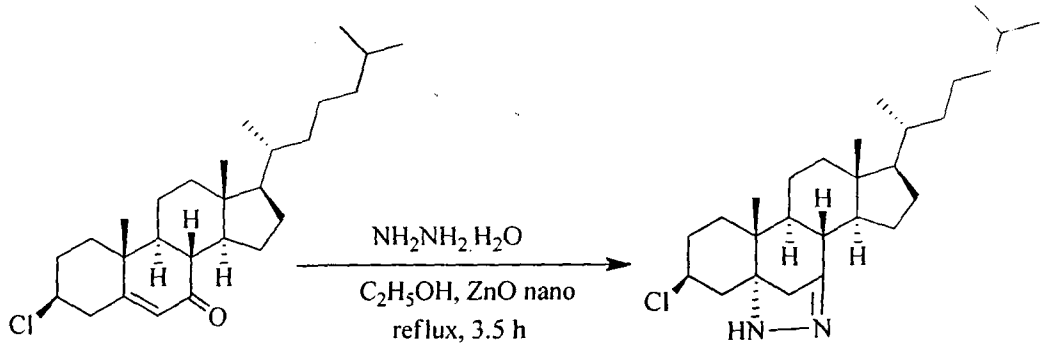
***Characterization of compound 71 as 3β-acetoxy-5α-cholestano-[5, 7- c d]-pyrazoline*:**

The elemental analysis of compound **71** corresponded to the molecular formula $C_{29}H_{48}N_2O_2$. The IR data provided evidence for the formation of the expected structure. The compound showed intense band in the region of 3270 cm^{-1} due to N-H stretching vibration. In addition, other important absorption bands at 1736 and 1655 cm^{-1} were attributed to C=O and C=N, respectively. Further evidence for the formation of compound **71** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited one-proton singlet at δ 5.3 for NH (exchangeable with D_2O). A one-proton broad multiplet centered at δ 4.7 was assigned to C3-αH (axial, W ½ = 15 Hz) and a sharp singlet for three acetoxy group protons appeared at 2.03. Angular and side-chain methyl protons were observed at δ 1.18 (C10-CH_3), 0.70 (C13-CH_3), 0.92 and 0.85 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 156 for *C*=N and 47 for *C*-N, in addition to the signals of cholestane series. The mass spectrum was also in good agreement with its molecular formula which exhibited strong molecular ion peak at *m/z* 456.

On account of the above evidences, the compound **71** can be suitably characterized as *3β-acetoxy-5α-cholestano-[5, 7- c d]-pyrazoline.*

Reaction of 3β-chlorocholest-5-en-7-one (69) with $NH_2NH_2.H_2O$: 3β-Chloro-5α-cholestano-[5, 7- c d]-pyrazoline (72):

Steroidal α,β-unsaturated ketone **69** in ethanol was allowed to react with hydrazine hydrate under reflux condition in the presence of ZnO nanoparticles for 3.5 h, after usual work up and recrystallization a single product **72**, m.p. 140-142 °C, was obtained.



(69) **(72)**

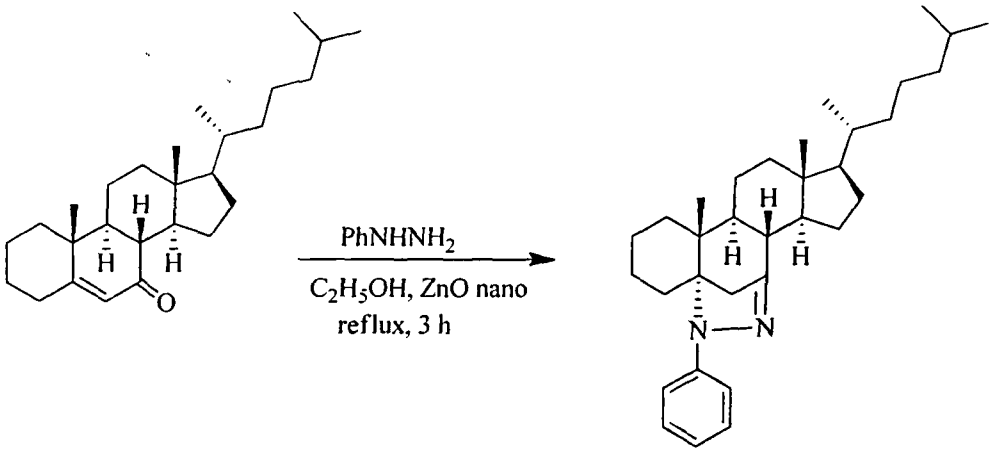
Characterization of compound 72 as 3β-chloro-5α-cholestano-[5, 7- c d]-pyrazoline:

The elemental analysis of compound **72** corresponded to the molecular formula $C_{27}H_{45}ClN_2$ (Beilstein positive). The IR data provided evidence for the formation of the expected structure. The compound showed intense band in the region of 3265 cm^{-1} due to N-H stretching vibration. In addition, other important absorption bands at 1650 and 776 cm^{-1} were attributed to C=N and C-Cl stretchings, respectively. Further evidence for the formation of compound **72** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited one-proton singlet at δ 5.4 for NH (exchangeable with D_2O). A one-proton broad multiplet centered at δ 3.9 was assigned to C3-αH (axial, $W_{1/2}$ = 15 Hz). Angular and side-chain methyl protons were observed at δ 1.19 (C10-CH_3), 0.75 (C13-CH_3), 0.92 and 0.86 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at 157 for *C*=N and 48 for *C*-N, in addition to the signals of cholestane series. The mass spectrum was also in good agreement with its molecular formula which exhibited a strong molecular ion peak at *m/z* 432/434.

On account of the above descriptive discussion, the compound **72** can be suitably characterized as *3β-chloro-5α-cholestano-[5, 7- c d]-pyrazoline.*

c d]-pyrazoline (73):

Steroidal α,β-unsaturated ketone **67** in ethanol was allowed to react with phenyl hydrazine under reflux condition in the presence of ZnO nanoparticles for 3 h. After usual work up and recrystallization from methanol a solid compound **73** was obtained, m.p. 185-187 °C.



(67) (73)

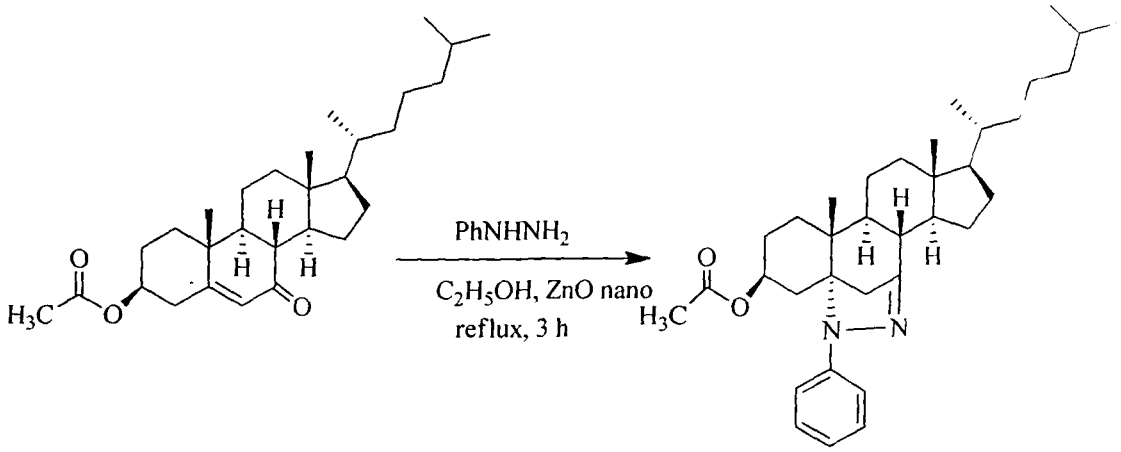
Characterization of compound 73 as 2'-phenyl-5α-cholestano-[5, 7- c d]-pyrazoline:

The elemental analysis of compound **73** corresponded to the molecular formula $C_{33}H_{50}N_2$. The selected diagnostic bands in IR spectrum of synthesized compound **73** provided useful information for determining its structure. The absorption bands at 3095, 1590 and 1406 cm^{-1} confirmed the presence of aromatic ring. In addition, other important absorption bands at 1640 and 1233 cm^{-1} were attributed to C=N and C-N, respectively. Further evidence for the formation of compound **73** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited signals at δ 7.34-7.32 (m, 2H), 7.2-7.1 (m, 2H), 6.8 (m, 1H) attributed to aromatic protons. Angular and side-chain methyl protons were observed at δ 1.12 (C10-CH_3), 0.71 (C13-CH_3), 0.92 and 0.85 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 156 for *C*=N and 48 for *C*-N, in addition of the signals of cholestane series. The mass spectrum was also in good agreement with its molecular formula which exhibited strong molecular ion peak at *m/z* 474.

On account of the above evidences, the compound **73** can be suitably characterized as *2'-phenyl-5α-cholestano-[5, 7- c d]-pyrazoline.*

***Reaction of 3β-acetoxycholest-5-en-7-one (68) with phenyl hydrazine: 3β-Acetoxy-2'-phenyl-5α-cholestano-[5, 7- c d]-pyrazoline (74)*:**

Steroidal α,β-unsaturated ketone **68** in ethanol was allowed to react with phenyl hydrazine under reflux condition in the presence of ZnO nanoparticles for 3 h. After usual work up and recrystallization from methanol a solid compound **74** was obtained, m.p. 180-182 °C.



(68) **(74)**

***Characterization of compound 74 as 3β-acetoxy-2'-phenyl-5α-cholestano-[5, 7- c d]-pyrazoline*:**

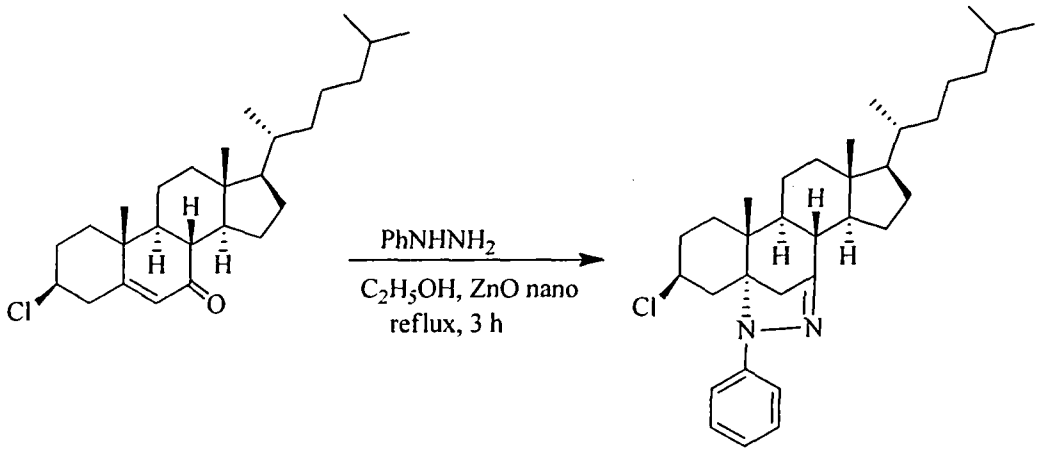
The elemental analysis of compound **74** corresponded to the molecular formula $C_{35}H_{52}N_2O_2$. The selected diagnostic bands in IR spectrum of the synthesized compound provided useful information for determining its structure. The absorption bands at 3130, 1564 and 1394 cm^{-1} confirmed the presence of aromatic ring. In addition, other important absorption bands at 1734 and 1630 cm^{-1} were attributed to C=O (ester) and C=N, respectively. Further evidence for the formation of compound **74** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited signals at δ 7.30-7.34 (m, 2H), 7.1-7.2 (m, 2H) and 6.9 (m, 1H) assigned to aromatic protons. A one-proton broad multiplet centered at δ 4.6 was assigned to C3-αH (axial, $W\,½$ = 15 Hz) and a sharp singlet for three acetoxy group protons appeared at 2.01. Angular and side-chain methyl protons were observed at δ 1.19 (C10-CH_3), 0.75 (C13-CH_3), 0.92 and 0.85 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure

signals of cholestane series. The mass spectrum was also in good agreement with its molecular formula which exhibited strong molecular ion peak at *m/z* 532.

On account of the above evidences, the compound **74** can be suitably characterized as *3β-acetoxy-2'-phenyl-5α-cholestano-[5, 7- c d]-pyrazoline.*

Reaction of 3β-chlorocholest-5-en-7-one (69) with phenyl hydrazine: 3β-Chloro-2'-phenyl-5α-cholestano-[5, 7- c d]-pyrazoline (75):

Steroidal α,β-unsaturated ketone **69** in ethanol was allowed to react with phenyl hydrazine under reflux condition in the presence of ZnO nanoparticles for 3 h. After usual work up and recrystallization from methanol a solid compound **75** was obtained, m.p. 170-172 °C.



(69) (75)

Characterization of compound 75 as 3β-chloro-2'-phenyl-5α-cholestano-[5, 7- c d]-pyrazoline:

The elemental analysis of compound **75** corresponded to the molecular formula $C_{33}H_{49}ClN_2$ (Beilstein positive). The selected diagnostic bands of IR spectrum of the product provided useful information for determining its structure. The absorption bands at 3129, 1590 and 1407 cm^{-1} confirmed the presence of aromatic ring. In addition, other important absorption bands at 1635 and 743 cm^{-1} were attributed to C=N and C-Cl, respectively. Further evidence for the formation of compound **75** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited signals at δ 7.31-7.32 (m, 2H), 7.1-7.2 (m, 2H), 6.8 (m, 1H), assigned to aromatic protons. A one-proton broad multiplet centered at δ 3.9 was assigned to C3-αH (axial, W ½ = 17 Hz). Angular and side-

chain methyl protons were observed at δ 1.19 (C10-CH_3), 0.75 (C13-CH_3), 0.92 and 0.85 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 157 for *C*=N and 49 for *C*-N, in addition of the signals of cholestne series. The mass spectrum was also in good agreement with its molecular formula which exhibited prominent molecular ion peak at *m/z* 508/510.

On account of the above evidences, the compound **75** can be suitably characterized as *3β-chloro-2'-phenyl-5α-cholestano-[5, 7- c d]-pyrazoline.*

Synthesis of catalyst

ZnO nanoparticles were biosynthesized under green conditions by using *C. albicans* as a green, reusable, nontoxic and inexpensive heterogeneous catalyst.

Characterization of catalyst

UV spectrophotometry study

The UV-vis absorption spectra findings demonstrate a novel technique for the preparation of ZnO nanoparticles (**Figure 1**), by dispersing ZnO nanoparticles in distilled water and using distilled water as the reference. An absorption peak focused at 381 nm (3.26 eV) was found.

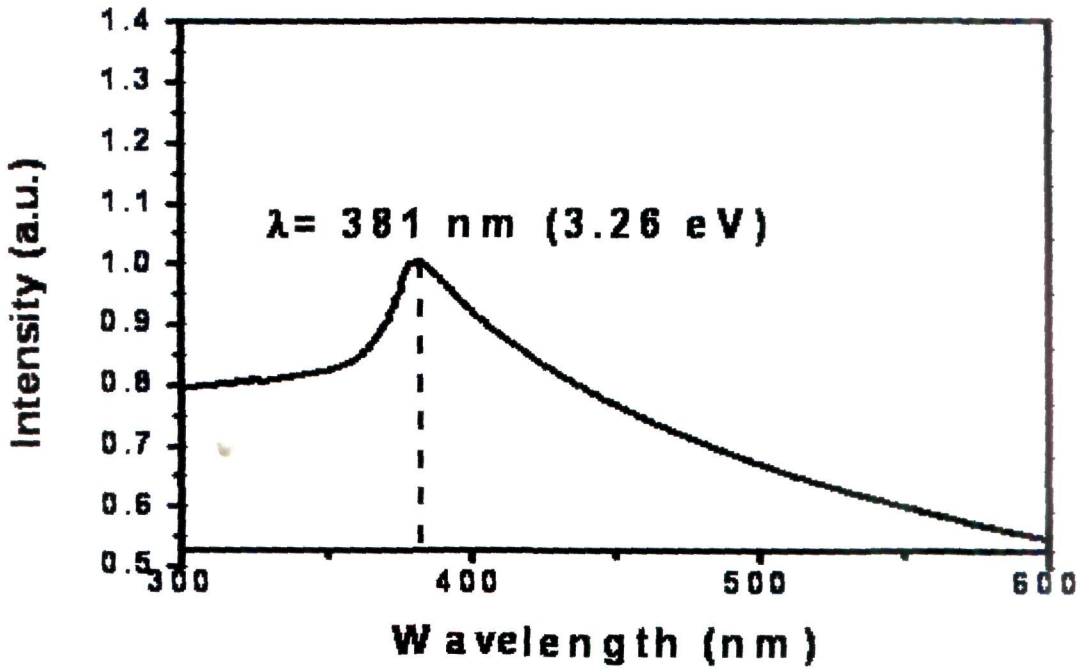


Figure 1. UV-visible spectrum of ZnO nanoparticles

XRD analysis

X-ray diffraction was taken in order to further confirm ZnO phase of the nanoparticles. The XRD patterns of the obtained ZnO nanoparticles are shown in **Figure 2**. Powder XRD of the product was carried out with Cu Kα radiation (λ = 0.1540 nm), employing a scanning rate of 0.02° s^{-1} and 2θ ranges from 20° to 80° for ZnO. All the peaks of XRD are very well matched with the hexagonal phase (wurtzite structure) by comparison with the data from JCPDS card No.89-7102 and no indication of a secondary phase. The strong and narrow diffraction peaks indicate that the product has well crystalline structure. The crystallite size of the nanoparticles was calculated using Debye Scherrer formula

$$D = K \lambda/\beta \text{ Cos } \theta,$$

Where, K is constant, λ is the wavelength of employed X-rays (1.54056 Å), β is corrected full width at half maximum and θ is Bragg's angle. The 2θ value from the equation comes out to be at 35.815 and therefore the calculated crystallite size of the powder particles is as about 25 nm.

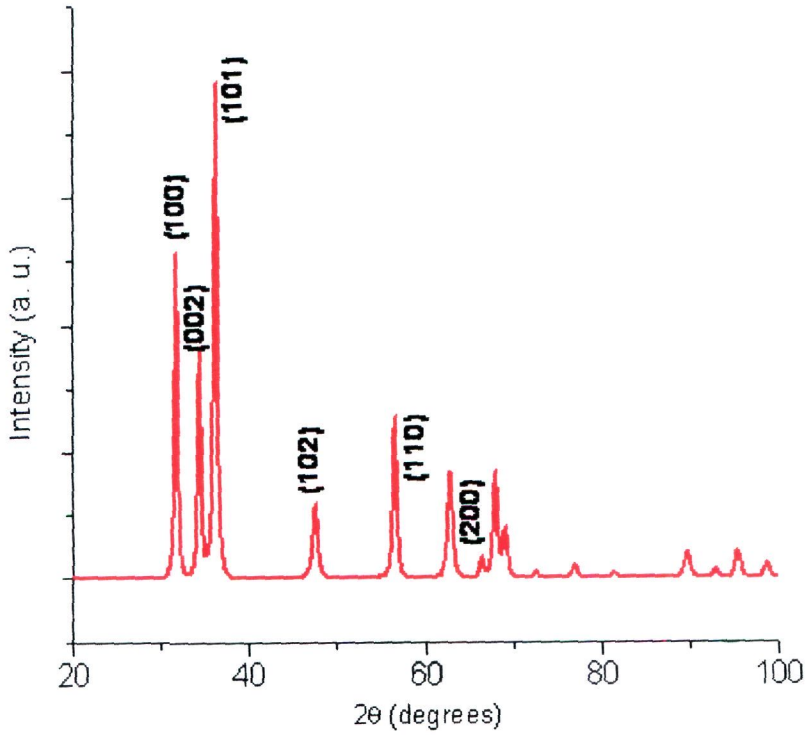


Figure 2. XRD image of the ZnO nanoparticles

SEM and TEM analysis

The conformation of the nanostructure morphology of ZnO particles comes from the analysis of SEM and TEM micrographs. SEM micrograph (**Figure 3**) showed the average size of nanoparticles between 15-25 nm.

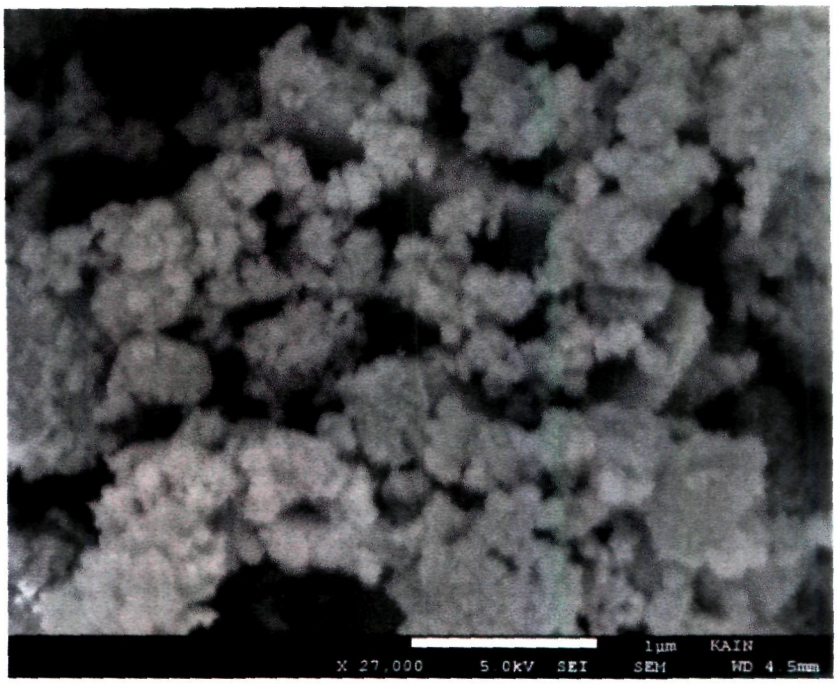


Figure 3. SEM image of the ZnO nanoparticles

The size and morphology of ZnO particles analyzed by TEM is represented in **Figure 4**. This image reveals that most of the ZnO nanoparticles are quasi-spherical and their diameter is about ~20 nm. This result is in accordance with the value calculated from the X-ray diffraction.

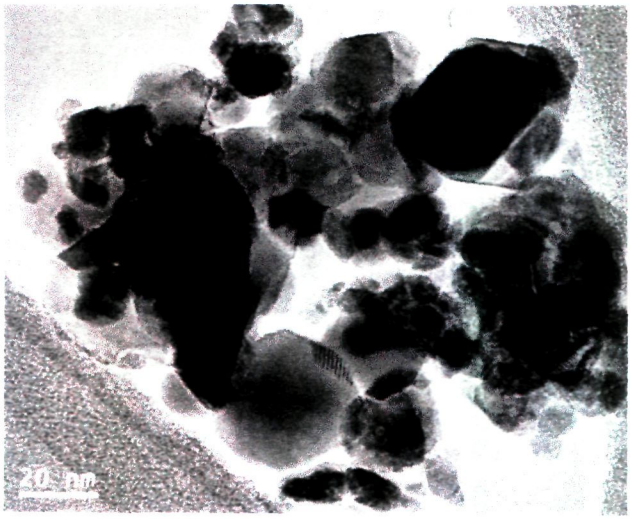


Figure 4. TEM image of the ZnO nanoparticles

Photoluminescence analysis

The PL spectrum of ZnO nanoparticles consists of two emission peaks (**Figure 5**). A weak deep-level emission at 2.3 eV in the visible range caused by a structural defect. The other peak in the UV range at 3.04 eV which can be explained by the direct combination of excitons through an exciton-exciton collision process and the lower energy peak in the asymmetric UV emission is associated with band-to-acceptor transitions due to the large binding energy of ZnO.

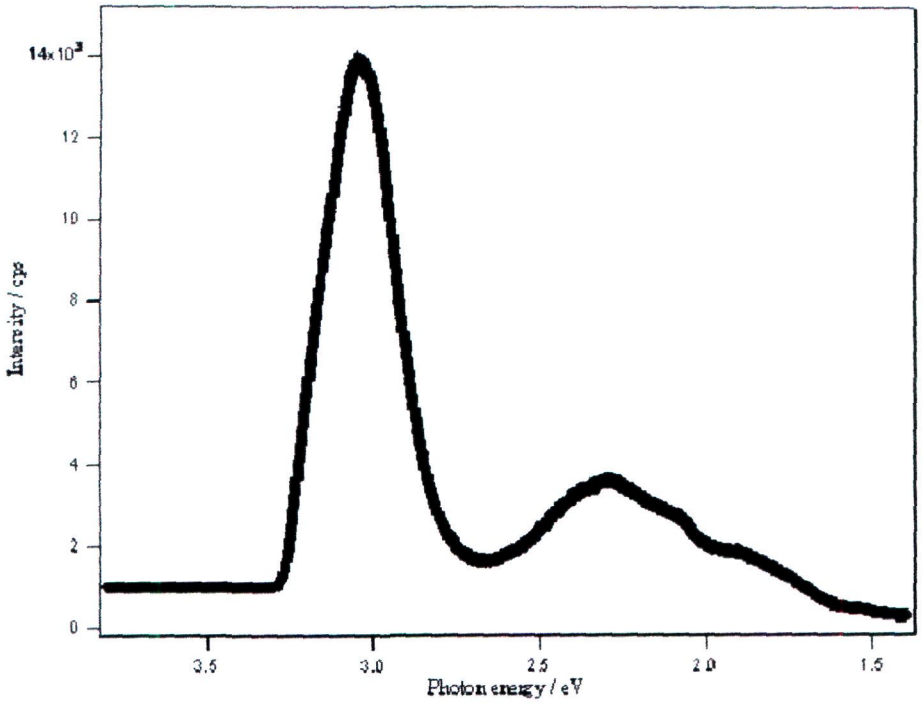


Figure 5. The PL spectrum of ZnO nanoparticles

Thermal stability

The differential thermal analysis and thermo gravimetric analysis (DTA/TGA) has been performed for the biosynthesis ZnO nanoparticles. TGA curve (**Figure. 6**) indicates that the weight loss starts at ~200 °C because of the evaporation of water, the major weight loss occurs between 340 to 550 °C, which is around 40 % of the original weight due to the removal and decomposition of organic groups present in the sample during the biosynthesis. No decomposition or reaction occurs at temperatures above 700 °C. The exothermic peak observed in the DTA plot as shown in **Figure 6** between 370-520 °C illustrates the maxima at ~435 °C which exemplifies the burn-out of organic composition. Apart from this, no other exothermic or endothermic peak is present in DTA curve.

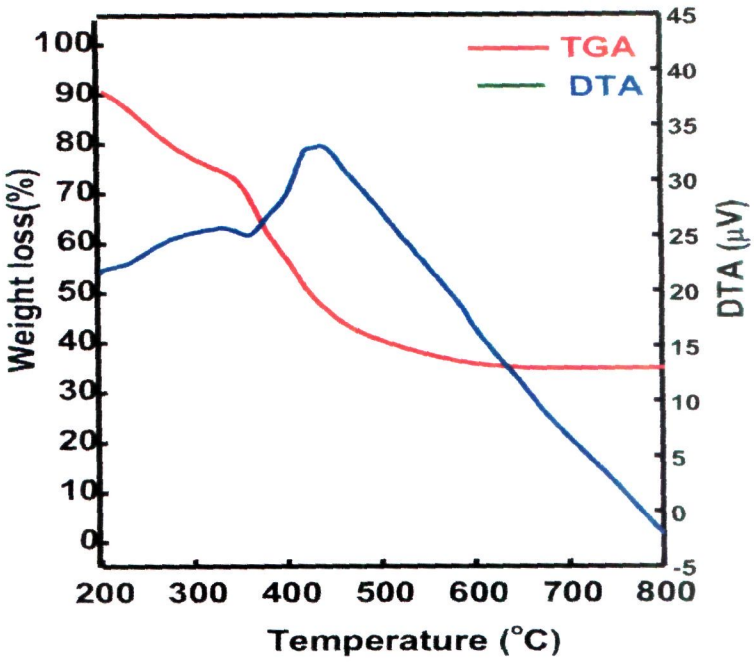


Figure 6. TGA and DTA curves of ZnO nanoparticles

The catalytic performance of ZnO in the synthesis of steroidal pyrazolines

As expected, the catalytic system is influenced by various reaction parameters, such as amount of the catalyst employed, effect of catalyst and solvent system. 3*β*-Acetoxycholest-5-en-7-one and hydrazine hydrate in ethanol were selected as model substrates for carrying out the optimization studies for synthesis of steroidal pyrazolines.

Catalytic Loading

The effect of catalyst loading on the synthesis of model reaction was investigated by varying the catalyst amount from 0 to10 mol % (**Table 1**).

Table 1. Effect of catalyst loading on the model reaction.

Entry	Catalysts (mol %)	Time (h)	Yield (%)[a]
1	2.5	4	30
2	5	3	80
3	7.5	3	80
4	10	3	80

[a]Yields are related to isolated pure products

It was found that 5 mol % of the catalyst was sufficient to get optimum yields in less reaction time while less than 5 mol % of the catalyst increased the reaction time. Use of more than 5

mol % of the catalyst did not show any profound effect on the reaction rate as well as the yield this may be attributed to the coagulation of ZnO nanoparticles which decreased the effective surface area of the catalyst.[43]

Effect of solvent

We then tried to screen the reaction in various organic solvents in order to optimize the reaction conditions using ZnO nanoparticles as catalyst (**Table 2**).

Table 2. Solvent screening for the model reaction.

Entry	Solvent	Yield (%)[a]
1	Acetonitrile	40
2	Methanol	75
3	DMSO	60
4	Toluene	30
5	Ethanol	80

[a]Yields are related to isolated pure products

The solvent screening experiments revealed that the reaction yield is dependent on the polarity and the coordinating ability of the solvents. The polar solvents afforded better yield than the nonpolar ones and the best result was obtained in ethanol in which ZnO nanoparticles catalyst worked most efficiently by phasing out of the desired product.

Recyclability of Catalyst

After completion of the model reaction in specified time, the catalyst was recovered by filtration, washed with dichloromethane and methanol and dried at 150 °C for 4 h and used for the subsequent cycle.[44] The results revealed that the catalyst exhibited good catalytic activity up to five cycles (**Table 3**).

Table 3. Recycling study of catalyst for the model reaction.

Entry	Time (h)	Yield (%)[a]
1	3	80
2	3	80
3	3	80
4	3	80
5	3	80
6	6	50

[a]Yields are related to isolated pure products

Studying the superiority of ZnO nanoparticles over some other metal oxide nanoparticles

Various catalysts were employed to evaluate the capability and efficiency of the catalyst (**Table 4**). Initially, the model reaction was performed in the absence of any catalyst and the reaction proceeded very slow and the expected product was in a very small quantity (entry 1) and when the model reaction was examined with MgO, CaO, Fe_2O_3 and Al_2O_3 nanoparticles using 5 mol% of each catalyst separately the reaction took longer time period for completion with lower yield of the product (entry 2-5). With ZnO nanoparticles the reaction was accelerated and yield of the desired product was maximum (**Table 4**, entry 6).

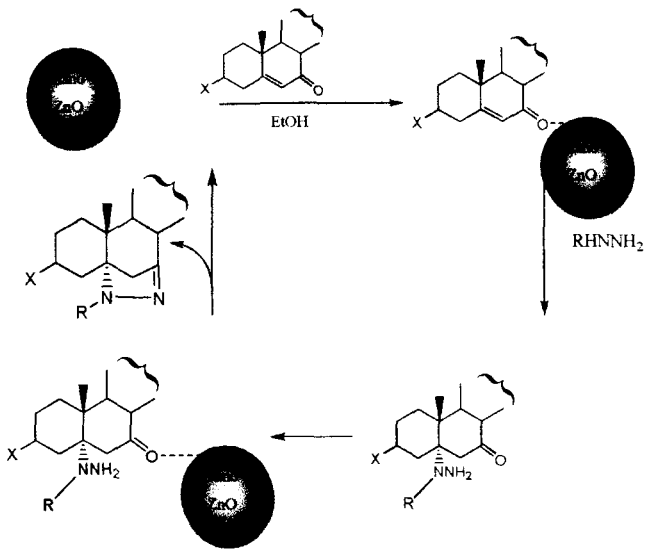
Table 4. The superiority of ZnO nanoparticles over some other metal oxide nanoparticles.

Entry	Catalysts	Time (h)	Yield (%)[a]
1	-	24	20
2	MgO	12	28
3	CaO	10	30
4	Fe_2O_3	6	32
5	Al_2O_3	6	53
6	ZnO	3	80

[a]Yields are related to isolated pure products

These observations may be explained by HSAB (hard and soft acid base) concept Unlike Zn^{2+} which is a borderline Lewis acid, all the rest of nanoparticles are classified as hard Lewis acids and the first step of the reaction is formation of intermediate by the interaction of Lewis acid catalyst with rather soft Lewis base unsaturated carbonyl group was assumed to be efficient for ZnO nanoparticles than the other.[45]

With these excellent results in our hand, the mechanism of the reaction can be proposed which involves Michael addition and then intramolecular cyclization catalyzed by ZnO nanoparticles as presented in **Scheme 1**. In first step, ZnO nanoparticles increase the electrophilicity of carbonyl group which in turn accelerate the Michael addition step. At the time of cyclization, the particular catalyst plays the key role where ZnO acts as a mild acid and activates the ketone group by polarization (through coordination) and hence facilitates the intramolecular nucleophilic attack by the NH_2 group leading to the ring closure which ultimately forms the final product and again the catalyst enters into the catalytic cycle.



Scheme 1. Proposed mechanism of formation of 5α-cholestano-[5, 7- *c d*]-pyrazoline derivatives catalyzed by ZnO nanoparticles.

Stereochemistry

The stereochemical assignation of C5-N bond has been established on the basis of mechanism as well as on ^{1}H and ^{13}C NMR spectral analysis of the compounds. During the course of reaction, the nucleophilic attack of N of the reagent at C-5 does occur preferably from less hindered (α) side because of the steric encumbrance imposed by axial (β) methyl group at C-10, resulting axial (α) position of C5-N bond and *trans* to C10-axial methyl group (A/B ring junction *trans*). The determination of ring fusion stereochemistry in angularly methylated six-membered ring compounds is not an easy task by either chemical or physical methods, NMR spectroscopy may, however, greatly aid in the solution of this problem. ^{13}C NMR values of C-19 and C-9 are strongly dependent on the ring fusion stereochemistry. The *cis* and *trans* steroids differ most significantly at C-19 and these signals will surely be of prime value to characterize the nature of the ring junction.[46] In all-*trans* steroids, the C-19 resonance is well separated from the majority of those of the other carbons. In the compounds **70-75**, C-19 chemical shift values were observed in the range of 16-18 ppm, which is consistent with values obtained for *trans* steroids[47] (A/B ring junction *trans*). Furthermore the half band width ($W_{1/2}$) values of C3-axial proton in the ^{1}H NMR spectrum of the synthesized compounds clearly suggest that A/B ring junction is *trans*.[48]

Experimental

General

Melting points were determined on a Kofler apparatus. The IR spectra were recorded on KBr pellets with Interspec 2020 FT-IR Spectrometer spectro Lab and values are given in cm^{-1}. ^1H and ^{13}C NMR spectra were run in $CDCl_3$ on a Bruker Avance II 500 NMR Spectrometer at 500 MHz and 125 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to the TMS (^1H NMR) and to the solvent signal (^{13}C NMR spectra). Mass Spectra were recorded on a JEOL D-300 Mass Spectrometer. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapors to check the homogeneity as well as the progress of reaction. Sodium sulfate (anhydrous) was used as a drying agent. The optical absorbance spectra were taken by using UV-vis double beam Perkin-Elmer LAMDA 35 spectrophotometer at room temperature in the wavelength range of 300-600 nm. X-ray diffraction (XRD) patterns of the nanoparticles were obtained at room temperature, with a step of 0.02°, using Bruker D8 ADVANCE X-ray diffractometer with Cu Kα radiation (λ=1.54178 Å) in the range of $0° \leq 2\theta \leq 100°$ at 40 kV. SEM images were obtained using a field emission scanning electron microscope (JSM-7600F, JEOL, Tokyo Japan) at an accelerating voltage of 15 kV and TEM images were obtained with ultra-high resolution FETEM (JEOL, JEM-2100F) at an accelerating voltage of 200 kV. Photoluminescence (PL) spectra were measured using a Cary Eclipse EL06063917 fluorescence spectrophotometer with a xenon arc lamp as the light source. The thermal studies were carried out using TGA/DTA- 60H instrument (SHIMADZU) at a heating rate of 20 °C min^{-1}.

***3β-Chlorocholest-5-ene*:**

Freshly distilled thionyl chloride (20 mL) was added gradually to cholesterol (25 g) at r.t. A vigorous reaction ensured with evolution of gaseous products. When the reaction slackened, the mixture was gently heated at temperature 50-60 °C on water bath for 1 h and then poured into water with constant stirring. The yellow solid thus obtained was filtered under suction and washed several times with ice-cold water and air dried. Recrystallization from acetone gave 3β-chlorocholest-5-ene (20 g), m.p. 95-96 °C (reported, m.p. 96-97 °C).[47]

***Cholest-5-ene*:**

3β-Chlorocholest-5-ene (10 g) was dissolved in warm amyl alcohol (230 mL) and sodium metal (20 g) was added in small portion to the solution with continuous stirring over a period of 8 h. The reaction mixture was warmed occasionally. When all the sodium metal was dissolved, methanol was added and the reaction mixture was poured into water, acidified with dilute HCl and then allowed to stand overnight. A white crystalline solid thus obtained

was filtered under suction and washed thoroughly with water and air dried. The crude material was recrystallized from acetone to provide cholest-5-ene as cubes (8.3 g), m.p. 88-89 °C (reported m.p. 89-91 °C).[42a]

***Cholest-5-en-7-one* (67):**

A solution of butyl chromate [*t-butyl* alcohol (60 mL), CrO_3 (20 g), acetic acid (84 ml) and acetic anhydride (10 mL)][47] was added at 0 °C to a solution of cholest-5-ene (8 g) in CCl_4 (150 mL), acetic acid (30 mL) and acetic anhydride (10 mL). The contents were refluxed for 3 h and then diluted with water. The organic layer was washed with sodium bicarbonate solution (5%) and water and then dried over anhydrous sodium sulfate. Evaporation of the solvents under reduced pressure furnished cholest-5-en-7-one (67) which was recrystallized from methanol (3.1 g), m.p. 128 °C (reported, m.p. 125-129 °C).[42a]

***3β-Acetoxycholest-5-ene*:**

A mixture of cholesterol (100 g), pyridine (150 mL, freshly distilled over KOH) and freshly distilled acetic anhydride (100 mL) was heated on a water bath for 2 h. The reaction mixture was poured into ice cold water and solid mass thus obtained was filtered under suction, washed thoroughly with water until free from pyridine and then air-dried. Recrystallization of the crude product from acetone gave 3β-acetoxycholest-5-ene (95 g), m.p. 114 °C (reported, m.p. 115-116 °C).[49]

***3β-Acetoxycholest-5-en-7-one* (68):**

A solution of butyl chromate [*t-butyl* alcohol (60 mL), CrO_3 (20 g), acetic acid (84 mL) and acetic anhydride (10 ml)][47] was added at 0 °C to a solution of 3β-acetoxycholest-5-ene (8 g) in CCl_4 (150 mL), acetic acid (30 mL) and acetic anhydride (10 mL). The contents were refluxed for 3 h and then diluted with water. The organic layer was washed with sodium bicarbonate solution (5 %) and water and then dried over anhydrous sodium sulfate. Evaporation of the solvents under reduced pressure furnished 3β-acetoxycholest-5-en-7-one (67) which was recrystallized from methanol (3.2 g), m.p. 161-163 °C (reported, m.p. 164 °C).[42b]

***3β-Chlorocholest-5-en-7-one* (69):**

A solution of butyl chromate [*t-butyl* alcohol (60 mL), CrO_3 (20 g), acetic acid (84 mL) and acetic anhydride (10 mL)][47] was added at 0 °C to a solution of 3β-chlorocholest-5-ene (8 g) in CCl_4 (150 mL), acetic acid (30 mL) and acetic anhydride (10 mL). The contents were refluxed for 3 h and then diluted with water. The organic layer was washed with sodium bicarbonate solution (5 %) and water and then dried over anhydrous sodium sulfate. Evaporation of the solvents under reduced pressure furnished 3β-chlorocholest-5-en-7-one

(**69**) which was recrystallized from methanol (3.4 g), m.p. 144 °C (reported, m.p. 144-145 °C).[42c]

General procedure for the synthesis of steroidal pyrazoline derivatives **(70-75):**

A mixture of α,β-unsaturated steroidal ketones (**67-69**) (1 mmol), hydrazine hydrate/ phenyl hydrazine (1 mmol) and ZnO nanoparticles catalyst (5 mol%), in ethanol (5 mL) was refluxed for 3-3.5 h. The progress of the reaction was followed by TLC. After completion of the reaction, the mixture was filtered to remove the catalyst and the filtrate was taken in diethyl ether, washed with water and dried over anhydrous sodium sulfate. Removal of solvent gave the crude product which was recrystallized from methanol to obtain the pure compounds (**70-75**).

5α-Cholestano-[5, 7- c d]-pyrazoline **(70)**

Yield (82%); m.p. 134-136 °C; Anal. Calcd for $C_{27}H_{46}N_2$: C, 81.34, H, 11.63, N, 7.03; found; C, 81.37, H, 11.66, N, 7.05; IR (KBr) ν cm^{-1} 3267 (NH), 1657 (C=N), 1268 (C-N); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.2 (s, 1H, NH), 2.32 (s, 2H, C6-H), 1.19 (s, 3H, C10-CH_3), 0.75 (s, 3H, C13-CH_3), 0.91 and 0.85 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 155, 65, 47, 46, 45, 43, 42, 41, 40, 39, 36, 35, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 22, 21, 19, 16; MS (EI): (*m/z*) 398 [M^+].

3β-Acetoxy-5α-cholestano-[5, 7- c d]-pyrazoline **(71)**

Yield (80%); m.p: 137-139 °C; Anal. Calcd for $C_{29}H_{48}N_2O_2$: C, 76.27, H, 10.59, N, 6.13; found; C, 76.24, H, 10.55, N, 6.9; IR (KBr) ν cm^{-1} 3270 (NH), 1736 (O*C*OCH$_3$), 1655 (C=N), 1265 (C-N), 1235 (C-O); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.3 (s, 1H, NH), 4.7 (m, 1H, C3α-H, *W* ½ = 15 Hz, A/B *trans*), 2.5 (s, 2H, C6-H), 2.03 (s, 3H, $OCOCH_3$), 1.18 (s, 3H, C10-CH_3), 0.70 (s, 3H, C13-CH_3), 0.92 and 0.85 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172 , 156, 74, 66, 47, 46, 45, 43, 42, 41, 40, 39, 36, 35, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 16. MS (EI): (*m/z*) 456 [M^+].

3β-Chloro-5α-cholestano-[5, 7- c d]-pyrazoline **(72)**

Yield (82%); m.p. 140-142 °C; (Beilstein positive); Anal. Calcd for $C_{27}H_{45}ClN_2$: C, 74.87, H, 10.47, N, 6.47; found; C, 74.85, H, 10.48, N, 6.44; IR (KBr) ν cm^{-1} 3265 (NH), 1650 (C=N), 1254 (C-N) , 776 (C-Cl); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.4 (s, 1H, NH), 3.9 (m, 1H, C3α-H, *W* ½ = 17 Hz, A/B *trans*), 2.65 (s, 2H, C6-H), 1.19 (s, 3H, C10-CH_3), 0.75 (s, 3H, C13-CH_3), 0.92 and 0.86 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159, 64, 60.2, 48, 47, 46, 43, 42, 41, 40, 39, 36, 35, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 19, 16; MS (EI): (*m/z*) 432/434 [M^+].

2'-Phenyl-5α-cholestano-[5, 7- c d]-pyrazoline (73)

Yield (70%); m.p. 185-187 °C; Anal. Calcd for $C_{33}H_{50}N_2$: C, 83.84, H, 10.62, N, 5.90; found; C, 83.87, H, 10.66, N, 5.92; IR (KBr) ν cm^{-1}1640 (C=N), 3095, 1590, 1406 (aromatic ring), 1233 (C-N); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.34-7.32 (m, 2H), 7.2-7.1 (m, 2H), 6.8 (m, 1H), 2.32 (s, 2H, C6-H), 1.12 (s, 3H, C10-CH_3), 0.71 (s, 3H, C13-CH_3), 0.92 and 0.85 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156, 147, 131, 130, 122, 115, 113, 65, 48, 47, 46, 43, 42, 41, 40, 39, 36, 35, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 16; MS (EI): (*m/z*) 474 [$M^{+\cdot}$].

3β-Acetoxy-2'-phenyl-5α-cholestano-[5, 7- c d]-pyrazoline (74)

Yield (80%); m.p. 180-182 °C; Anal. Calc. for $C_{35}H_{52}N_2O_2$: C, 78.90, H, 9.84, N, 5.62; found; C, 78.88, H, 9.82, N, 5.58; IR (KBr) ν cm^{-1} 3130, 1564, 1394 (aromatic ring), 1734 ($OCOCH_3$), 1630 (C=N), 1242 (C-N), 1239 (C-O); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.30-7.34 (m, 2H), 7.1-7.2 (m, 2H), 6.9 (m, 1H), 4.6 (m, 1H, C3α-H, W ½ = 15 Hz, A/B *trans*), 2.32 (s, 2H, C6-H), 1.19 (s, 3H, C10-CH_3), 0.75 (s, 3H, C13-CH_3), 0.92 and 0.85 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172, 156, 145, 130, 129, 120, 116, 114, 74, 66, 48, 47, 46, 43, 42, 41, 40, 39, 36, 35, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 16; MS (EI): (*m/z*) 532 [$M^{+\cdot}$].

3β-Chloro-2'-phenyl-5α-cholestano-[5, 7- c d]-pyrazoline (75)

Yield (76%); m.p. 170-172 °C; (Beilstein positive); Anal. Calc. for $C_{33}H_{49}ClN_2$: C, 77.84, H, 9.70, N, 5.50; found; C, 77.88, H, 9.72, N, 5.54; IR (KBr) ν cm^{-1} 3129, 1590, 1407 (aromatic ring), 1635 (C=N), 1235 (C-N), 743 (C-Cl); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.31-7.32 (m, 2H), 7.1-7.2 (m, 2H), 6.8 (m, 1H), 3.9 (m, 1H, C3α-H, W ½ = 17 Hz, A/B *trans*), 2.32 (s, 2H, C6-H), 1.19 (s, 3H, C10-CH_3), 0.75 (s, 3H, C13-CH_3), 0.92 and 0.85 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157, 146, 128, 126, 119, 115, 114, 64, 60, 49, 47, 46, 43, 42, 41, 40, 39, 36, 35, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 19, 16; MS (EI): (*m/z*) 508/510 [$M^{+\cdot}$].

Biological synthesis of ZnO nanoparticles

The ZnO nanoparticles were prepared by biological methods. *C. albicans* cells were allowed to grow as a suspension culture in sterile distilled water containing nutrient broth media for 24 h and treated with 1.0% NaCl. 25 ml of culture was taken and diluted four times by adding 75 mL of sterile distilled water containing nutrients. This diluted culture solution was again allowed to grow for another 24 h. 20 mL aqueous solution of 1 mM zinc oxide (ZnO) was added to the culture solution and it was kept at 30 °C for 24 h until white deposition starts to appear at the bottom of the flask, indicating the initiation of

transformation. The culture solution was cooled and allowed to incubate at room temperature in the laboratory ambience. After 15 h, the culture solution was observed to have distinctly makeable coalescent white clusters deposited at the bottom of the flask. The reaction mixture was subjected to centrifugation for 15 min. The resulting pellet was stored at dark until further used.

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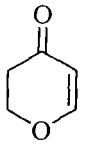
CHAPTER-2

Steroidal pyranones

Theoretical

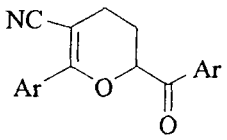
The pyranone ring system (**1**) consists of unsaturated six membered ring containing ethereal oxygen and ketonic group. Steroidal derivatives having pyranone ring fused with steroidal nucleus are termed as steroidal pyranones.



(1)

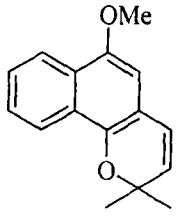
Pyranones and corresponding derivatives have been the subject of much research due to their importance in the field of synthetic and medicinal chemistry such as antiviral,[1] stimulant,[2] anticonvulsant,[3] miticidal activities.[4] Inspite of being building blocks in the field of synthetic and medicinal chemistry, pyranone rings are highly susceptible to nucleophillic attack at the electrophillic centres (C-2, C-4 and C-6) and a variety of synthetic approaches for the preparation of arenes and heteroarenes.[5] Furthermore the development of new methods with great efficiency, convenient procedures and better yields is of interest. The theoretical part of this chapter includes the recent reports of synthesis of pyranones by different workers.

Christ *et al.*[6] reported that dihydro-1,4-pyran (**2**) are cyclic ethers of aldehydes or ketones, and these substances might be expected to behave as vinyl ether.



(2)

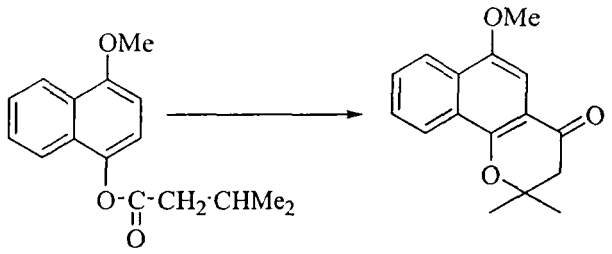
Livingstone and his coworkers [7] isolated and characterized Lapachenole a substance present in the wood of "*Brazilian white peroba*". Its structure has been elucidated as 4'-methoxy-6,6-dimethylnaphtho (1',2'-2,3) pyran (**3**) and confirmed by the total synthesis of its dihydroderevative.



(3)

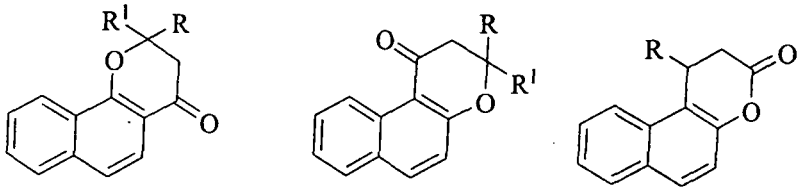
Later they synthesized 6-methoxy-2,2-dimethyl-7,8-benzochroman-4-one (**5**) from the esterification of 4-methoxy-l-naphthol with 3,3dimethylacryloyl chloride by the Fries

rearrangement of 4-methoxy-l-naphthyl-3,3-dimethylacrylate (**4**) in absence of solvent followed by acidic isomerization and ring closures.

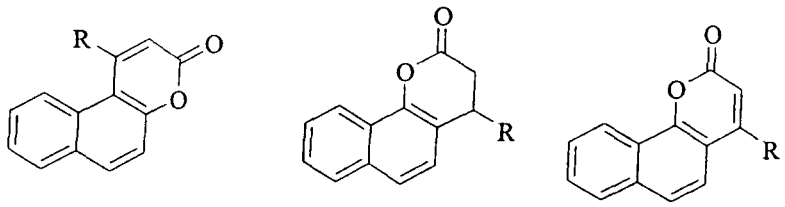


(**4**) (**5**)

Anjaneyulu *et al.*[8] reported the synthesis of 7,8-benzo 2,2-dimethyl chromanones (**6**, **7**) [R = R^1 = CH_3] and 5,6-benzo-3,4-dihydro-4-phenyl coumanns (**8-11**) [R = C_6H_5] by the reaction of 1-naphthol and 2-naphthol with $SbCl_3$, polyphosphoric acid, H_2SO_4 and $ZnCl_2$.

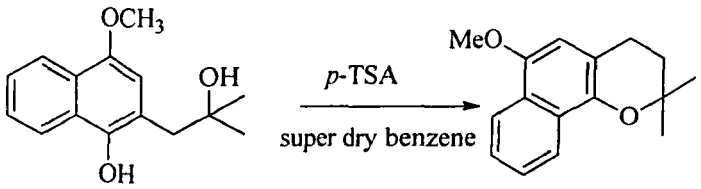


(**6**) (**7**) (**8**)



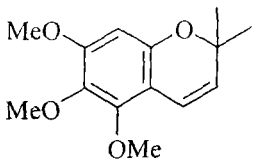
(**9**) (**10**) (**11**)

Gupta *et al.*[9] reported the direct synthesis of dihydro lapachenole (**13**) from l-hydroxy-4-methoxy-2-(3-hydroxy-3-methylbutyl) naphthalene (**12**).

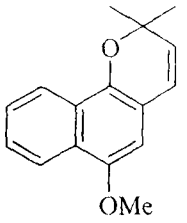


(**12**) (**13**)

Hlubucek *et al.*[10] reported the synthesis of evodionol methyl ether (**14**) and lapachenole (**15**) from 2,6-dimethoxy-4-hydroxyacetophenone and 4-methoxy-l-naphthol, respectively.

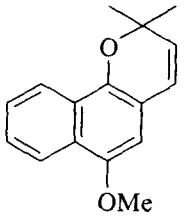


(14)

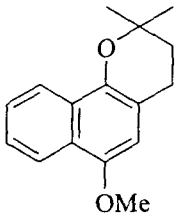


(15)

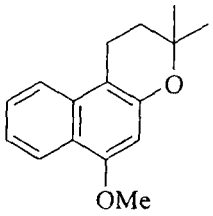
Khanna and co-workers[11] synthesized 2,2-dimethyl-naphtho [1,2- *b*] pyran (**16**) by the treatment of 1-naphthol with 2-chloro-2-methylbut-3-yne followed by the cyclization with N, N-dimethylanilme. Catalytic reduction of 2,2-dimethylnaphtho [1,2- *b*] pyran (**16**) gave 3,4-dihydro-2,2-dimethylnaphtho [1,2- *b*] pyran (**17**). Similarly the corresponding isomers 2, 2-dimethylnaphtho [2,3- *b*] pyran (**19**) and 3,4-dihydro-2,2-dimethylnaphtho [2,3- *b*] pyran (**18**) have been synthesized starting from 2-naphthol.



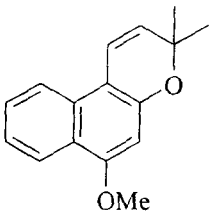
(16)



(17)

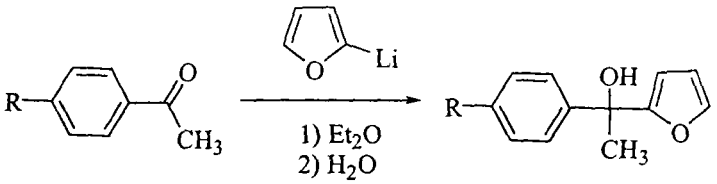


(18)

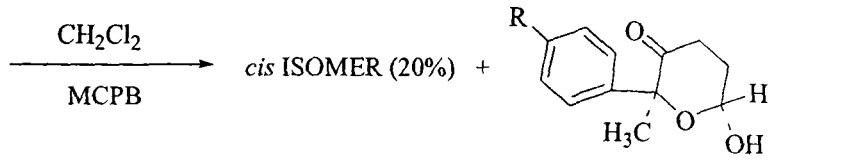


(19)

Georgiadis *et al.*[12] synthesized 2-methyl-2-[*p*-benzenesulphonyl) phenyl]-6-hydroxy-2*H*-pyran-3(6*H*)-one (**23**) from *p*-(benzenesulphonyl) acetophenone (**20**).



(20) (21)

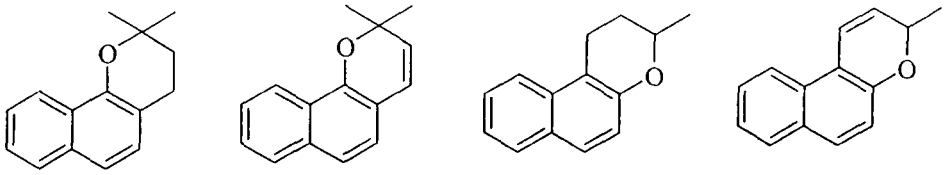


trans ISOMER (80%)

(22) (23)

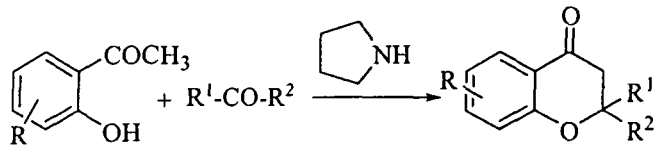
Ahluwalia and co-workers[13] synthesized 2,2-dimethyl-2*H*-naphtho[1,2- *b*] pyran (**25**) by the reduction of 3,4-dihydro-2,2-dimethyl-2*H*-naphtho [1,2- *b*] pyran (**24**) with DDQ or NBS. They also reported the synthesis of 1,2-dihydro-3,3-dimethyl-

3*H*-naphtho [2,l- *b*] pyran (**27**) and 3,3-dimethyl-3*H*-naphtho [2,l- *b*] pyran (**26**) from 2-naphthol.



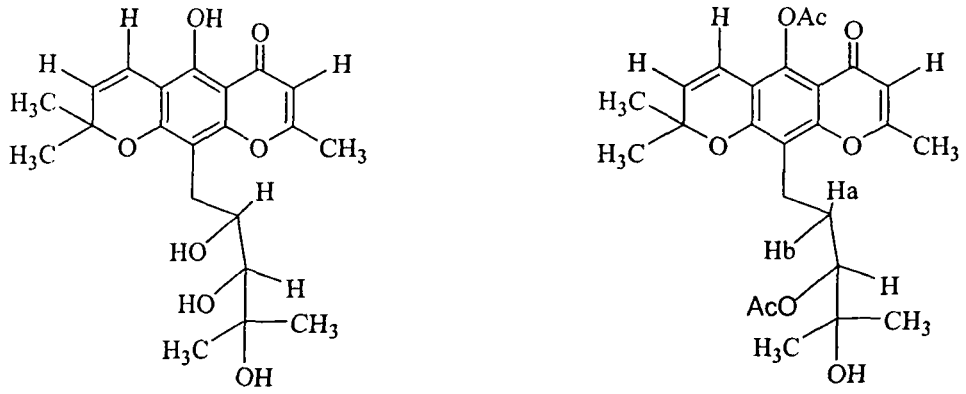
(24) **(25)** **(26)** **(27)**

Kabbe and Widdig[14] reported the synthesis of 4-chromanones (**30**) by the condensation of *o*-hydroxy acetophenones (**28**) with aliphatic carbonyl compounds (**29**) in presence of pyrrolidine.



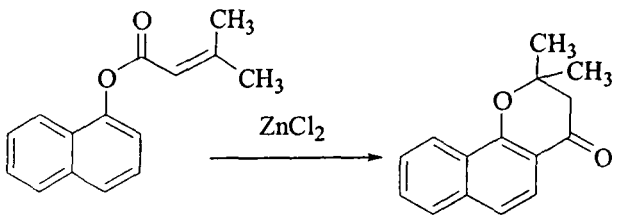
(28) **(29)** **(30)**

Suwanborirux *et al.*[15] isolated some new chromones (**31**) and (**32**) from 10% aq MeOH extract of twigs and leaves of *Spathelia sorbifolia*.



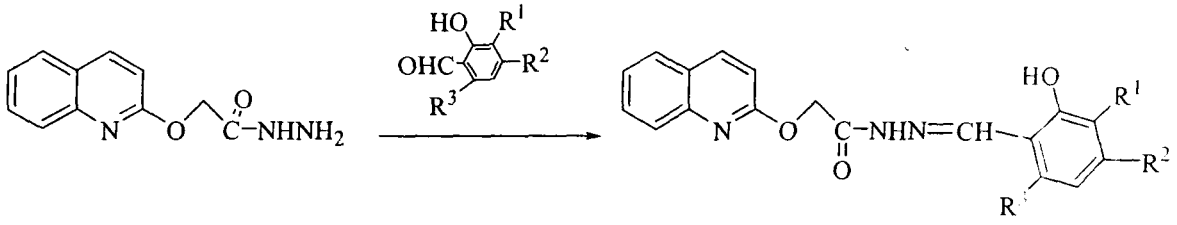
(31) **(32)**

Tanden *et al.*[16] reported the synthesis of 2,3-dihydro-2,2-dimethyl-4*H*-naphtho [1,2- *b*]-pyran-4-one (**34**) by the one-step cyclization of 1-(3-methyl-2-butenoyloxy)-naphthalene (**33**) with $ZnCl_2$.

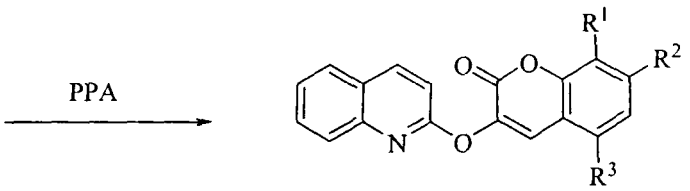


(33) **(34)**

Kidwai and co-workers[17] synthesized 3-(4'-methylquinolino-2'-yloxo)-2*H*-benzopyran-2-ones (**37**) [R^1 = CH_3, OCH_3, H, R^2 = CH_3, OCH_3, H, R^3 = OCH_3, CH_3, H] by the reaction of (4'-methylquinolino-2'-yloxo) acetic acid hydrazide (**35**) with 2-hydroxy-4-substituted benzaldehyde followed by cyclization in presence of PPA.

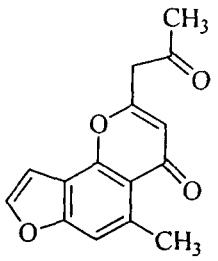


(**35**) (**36**)



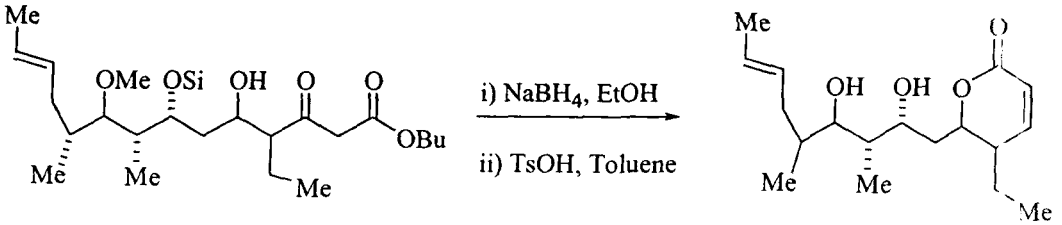
(**37**)

Speranza *et al.*[18] isolated furoaloesone a new 2-acetonyl-5-methyl-4*H*-furo [2,3- *b*][l]benzopyran-4-one (**38**).



(**38**)

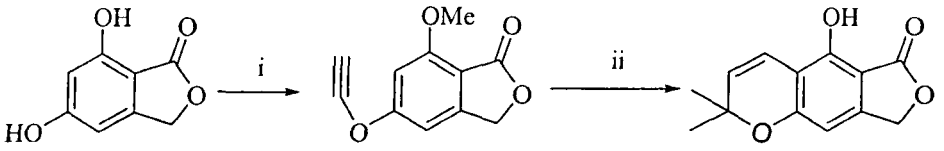
Nelson *et al.*[19] reported that *β* keto ester (**39**) undergoes reduction with $NaBH_4$ followed by the reaction with TsOH to give 2-pyranone unit (**40**).



(**39**) (**40**)

Mali *et al.*[20] reported that the dihydroxy phthalide (**41**) was monoprop-2-ynylated using 3-chloro-3-methylbut-1-yne in DMF solution in the presence of

K_2CO_3, KI and CuI to give the prop-2-ynyl ether (**42**), which on heating in N,N-dimethylanline solution provided salfredin B_{11} (**43**), which had pyran ring.

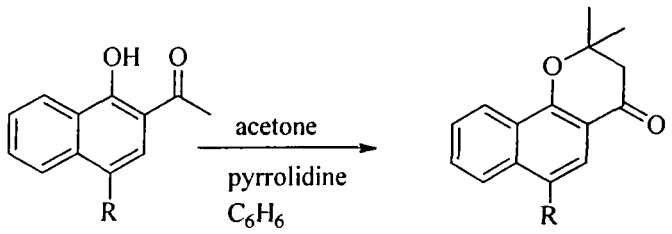


(41) **(42)** **(43)**

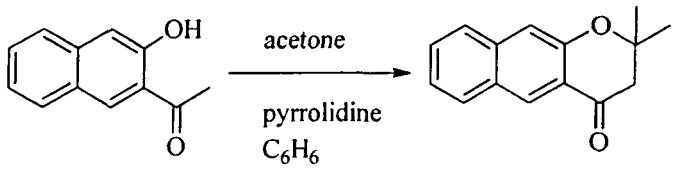
i) 3-Chloro-3-methylbut-1-yne, K_2CO_3, KI, CuI, DMF, 60 °C, 4h.

ii) $PhNMe_2$, 210 °C, 6h or MWI, 3min.

Paradkar *et al.*[21] synthesized naturally occurring angular naphthopyrans (**45**) starting from 2-acetyl-1-naphthol derivatives (**44**) [R = OMe, H] and linear naphthopyran (**47**) from 3-acetyl-2-naphthol (**46**).

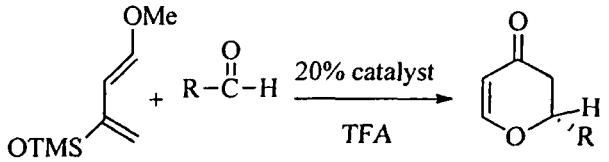


(44) **(45)**



(46) **(47)**

Feng *et al.*[22] reported the synthesis of *chiral* (*R*)-2-phenyl-2,3-dihydro-4*H*-pyran-4-one (**50**) from aldehyde (**49**) [R = Ph, 4-$MeOC_6H_4$, 4-MeC_6H_4, etc.] and Danishefsky's diene (**48**) using *chiral* H_8-BINOL-Ti.

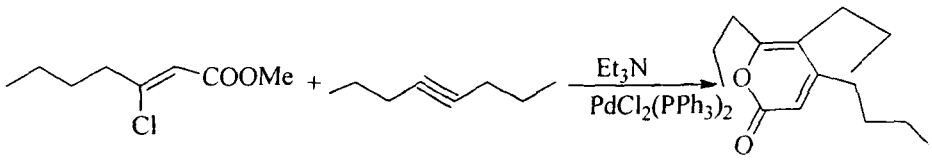


(48) **(49)** **(50)**

Hepworth *et al.*[23] synthesized 2*H*-1-benzopyran (**52**) from electrocyclization of aryl propagaryl ethers (**51**) which were readily prepared from phenols and alkynols.

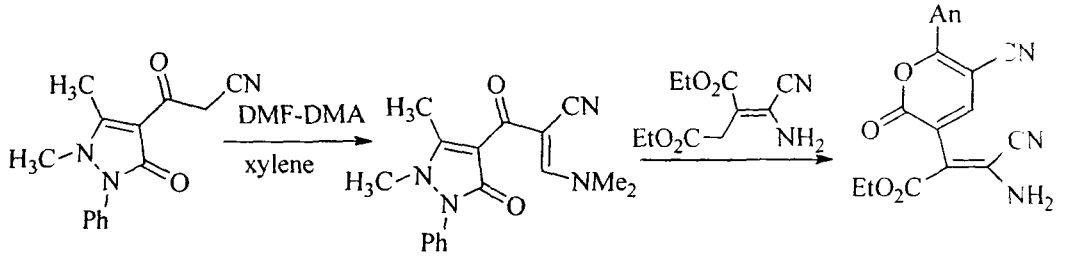
(51) (52)

Tanaka and Hua[24] prepared 2*H*-pyranone (**55**) by the palladium catalyzed reaction of internal alkynes (**54**) with (*Z*)-3-chloro-2-heptenaoate (**53**) in presence of Et_3N.

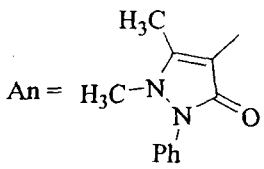


(53) (54) (55)

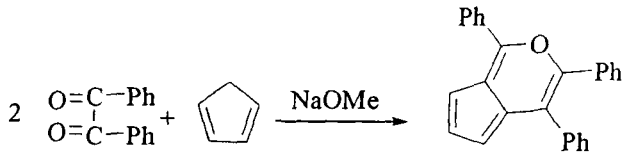
Elmaati and his co-worker[25] synthesized pyranone derivative (**58**) by the reaction of 3-dimethylamino-2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazole-4-carbonyl) acrylonitrile (**57**) with ethyl cyanoacetate dimer.



(56) (57) (58)

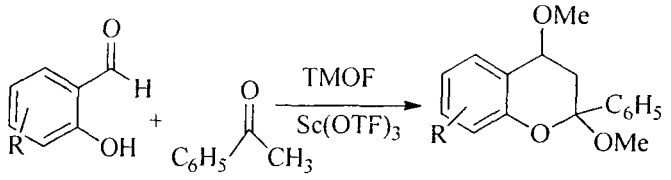


Banciu *et al.*[26] reported the preparation of 2,3,7-triphenylcyclopenta [*c*] pyran (**61**) from 1,2-diphenylethanedione (benzyl) (**59**) and cyclopentadiene (**60**).



(59) (60) (61)

Yadav *et al.*[27] synthesized 2,4-dialkoxy-2-aryl-3,4-dihydro-2*H*-1-benzopyrans (**67**) by the reaction of salicyldehydes (**65**) with acetophenones (**66**) in presence of tri methyl orthoformate and scandium triflat (catalytic amount) at ambient temperature

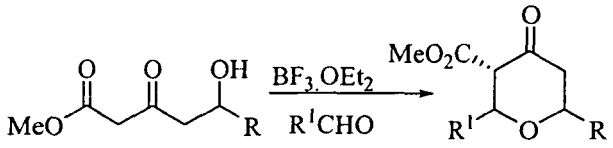


(65) (66) (67)

R = H, 3-OMe, 5-Cl

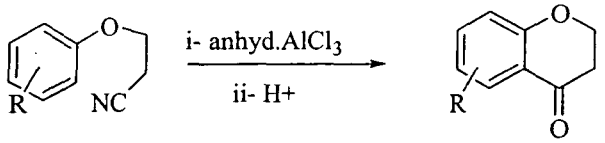
Ar = C_6H_5, 2-Naphthyl, 4-BrC_6H_4

Clarke and Martin[28] obtained single diastereomer of highly substituted tetrahydropyran-4-one (69) [R = hexyl, i-Pr, Ph, R^1 = Ph, Pr, hexyl] from *β*-ketoester (68).



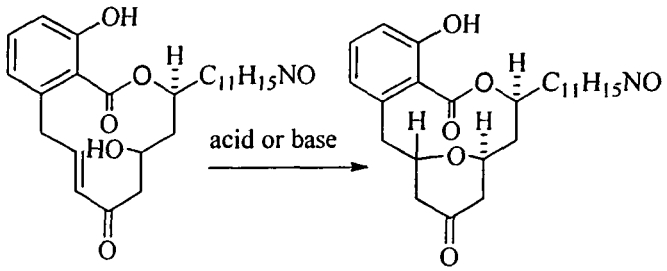
(68) (69)

Autino and co-workers [29] synthesized chromanones (71) by the cyclization of 3-aryl propiononitriles (70) by using $AlCl_3$ in the molar ratio of 1:1 in absence of solvent. [R = H, 6-CH_3, 6-OCH_3]



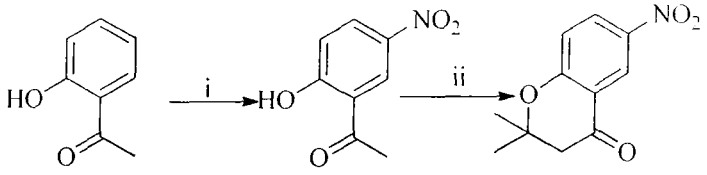
(70) (71)

Rizzacasa *et al.*[30] synthesized salicylihalamide a novel metabolite isolated from western Australian Marine sponge a pyranone unit (73) from *Apicularen* A (72).



(72) (73)

Peuli *et al.*[31] prepared 3',4'-dihydro-6'-nitro1*spiro* [cycloheptane-1',2(2'-*H*)[l] benzopyran]-4'-one (76) by the nitration of 2-hydroxyacetophenone (74) and subsequent treatment with appropriate carbocyclic ketone in presence of pyrrolidine.

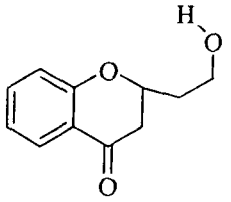


(74) (75) (76)

i) Conc. HNO_3, AcOH

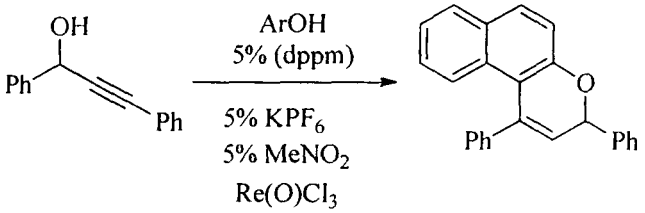
ii) Carbocyclic Ketone, pyrrolidine, toluene, reflux.

Kang *et al.*[32] isolated various strains from the roots of *Mimosa pudica* and one strain was identified as 2-hydroxymethyl-chroman-4-one (77).

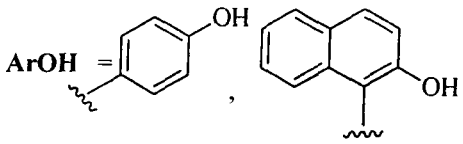


(77)

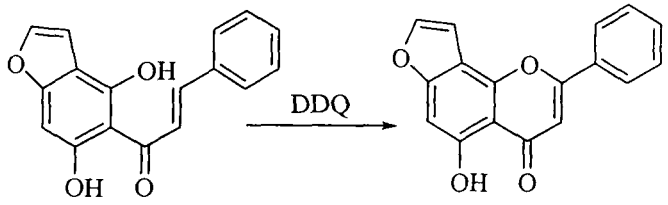
Toste *et al.*[33] reported the formation of benzopyrans (79) by the propargylation of phenols (78) catalyzed by a cationic ruthenium complex or protic acid.



(78) (79)

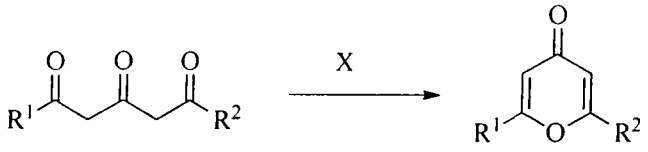


Islam *et al.*[34] prepared 8-hydroxy-5-phenyl-furo [2,3- *h*]benzo(*b*)pyran-7-one (81) by reaction of (*E*)-1-(4,6-dihydroxybenzofuran-5-yl)-3-phenylprop-2-en-1-one (80) with DDQ. The products were tested for anti bacterial effects.



(80) (81)

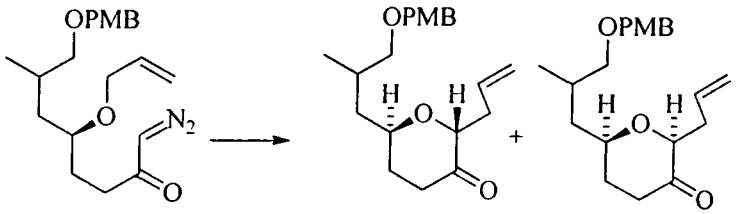
Yachevskii *et al.*[35] described the preparation of 2,6-bis-polyfluroalkyl-4*H*-pyran-4-ones (**83**) [R^1 = CHF_2, CF_3, C_2H_4H, C_4H_9, R^2 = CHF_2, CF_3, C_2F_4H] by dehydration of bis-polyfluoroalkyl containing 1,3,5-triketones (**82**).



(**82**) (**83**)

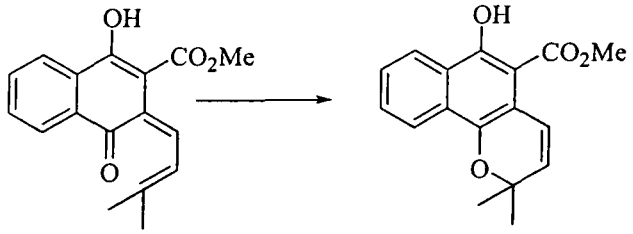
X = H_2SO_4, PPA, HCl/MeOH, $(Me_3SiO)_3$ PO, $(EtPO_2)_n/CHCl_3$.

Yakura and co-workers[36] reported the stereoselective synthesis of 2,3-disubstituted-3-pyranone (**85**) as a major product (82 %) and (**86**) by copper catalyzed oxonium ylide formation-[2,3] shift of (5*S*, 7*R*)-5-allyloxy-1-diazo-8-(*p*-methoxy-benzyloxy)-7-methyl-2-octanone (**84**).



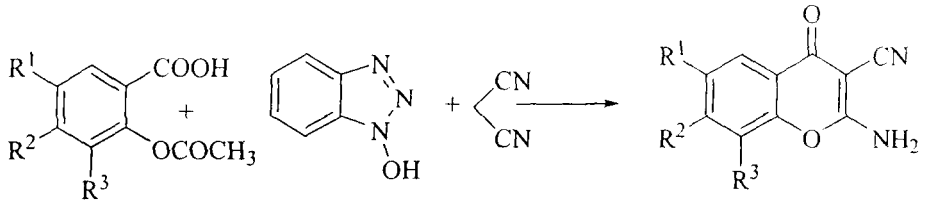
(**84**) (**85**) (**86**)

Trauner and co-worker[37] reported the syntehsis of mollugin a chromene (**88**) from 6π - electrocyclization of vinyl *o*-quinonemethide (**87**).



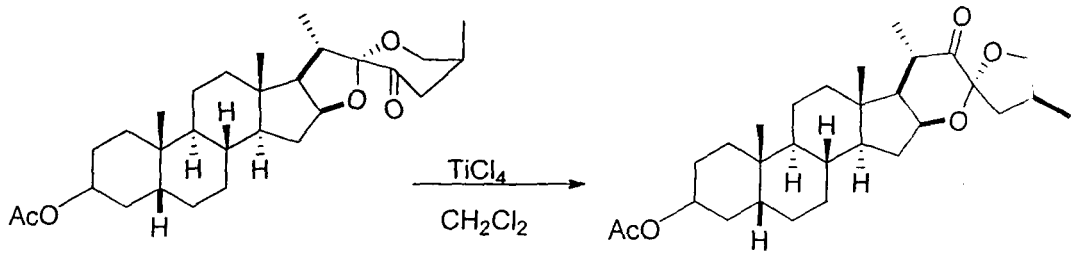
(**87**) (**88**)

Markopoulou *et al.*[38] reported the preparation of 2-amino-4-oxo-4*H*-chromene-3-carbonitrile (**92**) through a typical reaction of functionalized acetyl salicylic acid (**89**) with N-hydroxybenzotriazole (**90**) and malononitrile (**91**) [R^1 = H, Cl, CH_3, OCH_3, R^2 = H, OCH_3, R^3 = H, OCH_3].

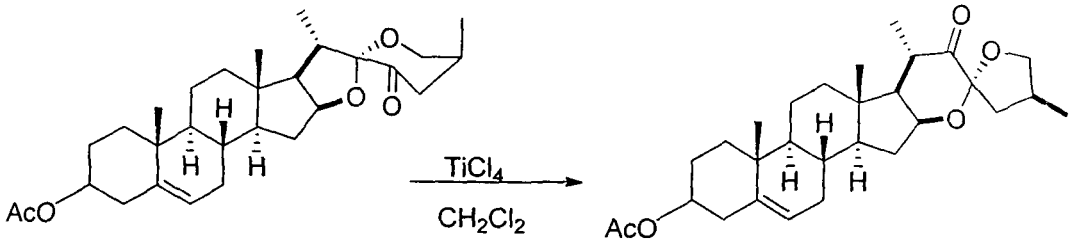


(89) (90) (91) (92)

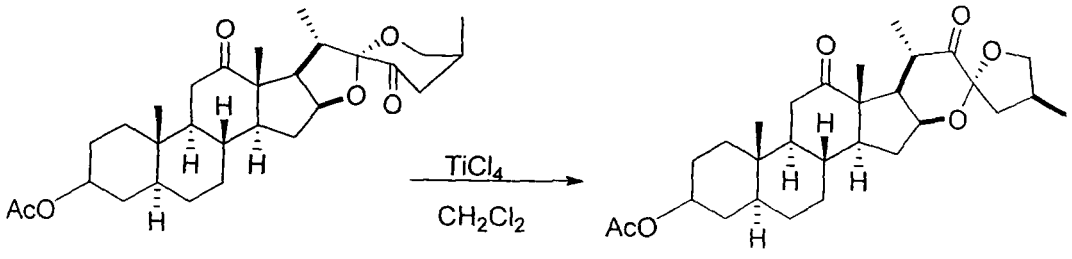
López *et al.*[39] obtained steroidal pyranone derivatives **94**, **96** and **98** from **93**, **95** and **97**, respectively.



(93) (94)

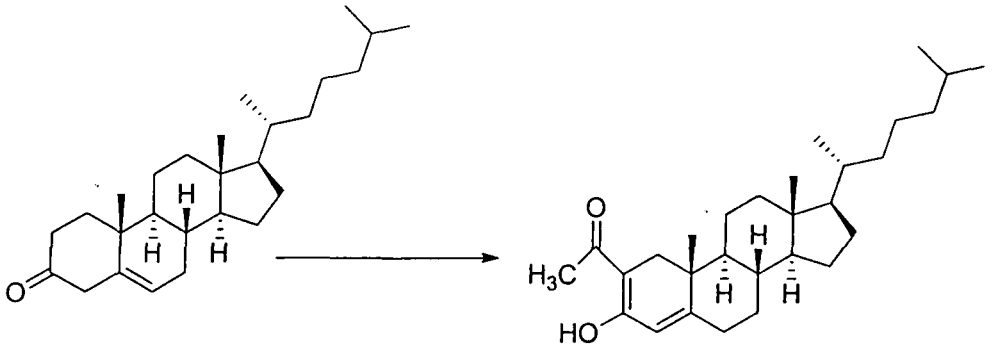


(95) (96)

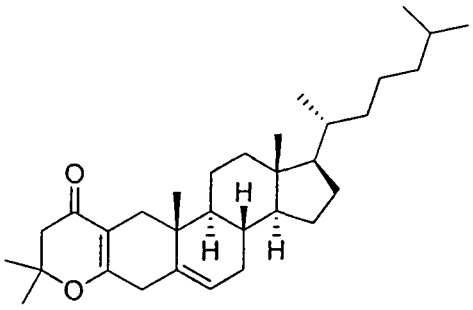


(97) (98)

2′,2′-dimethyl-cholesta-4,6-dien[7,6- *b*]-pyran-4′-one (**101**) was prepared by the cyclization of 2-acetylcholesta-2,4-dien-3-ol (**99**) with pyrrolidine, dry benzene and dry acetone using Dean Stark separator through conventional heating.[40]



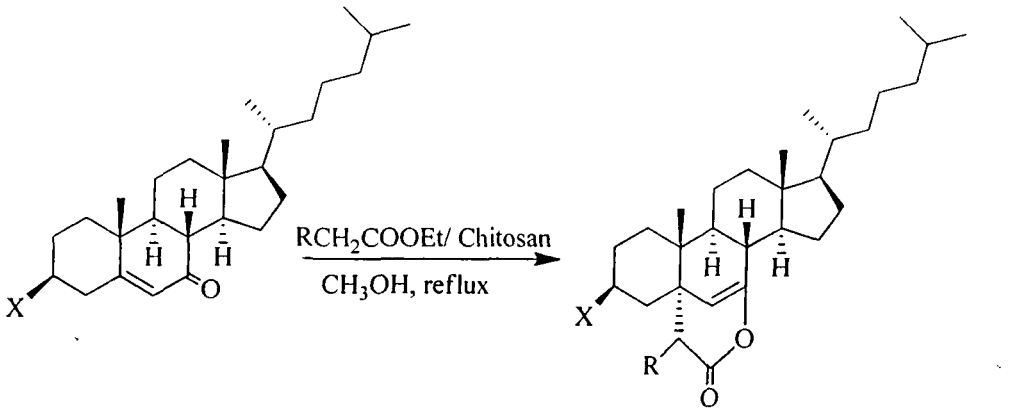
(99) **(100)**



(101)

Discussion

The importance of heterocyclic compounds has long been recognized in the field of synthetic organic chemistry and uses of pyranones are well established. Especially after the discovery that some pyranones are associated with a variety of physiological properties.[41] They are also used in perfumes, cosmetics and pharmaceutical industry. Pyranone moiety as also present in number of natural products including flavanoids, that interact with various enzymes and receptor system of pharmacological significance.[41] Many pyranone derivatives have been found to possess a broad spectrum of biological activities such as antitumor,[42]antiHIV,[43]antimicrobial,[44]antifungal,[45] and antioxidant.[46] Inspire of being important in medicinal chemistry, synthesis and reactions of pyranones have been widely investigated and ulilized for the preparation of multitude of heterocyclic compounds and may also be used as intermediates in the manufacture of compounds of physiological importance. In view of these reports and in continuation of our previous work[47] in steroidal chemistry, we have developed a new, highly efficient method for the synthesis of substituted steroidal pyranones (**105-110**) using chitosan as heterogeneous, basic and green catalyst. The substrates selected for initial studies include cholest-5-en-7-one (**102**), 3β-acetoxycholest-5-en-7-one (**103**) and 3β-chlorocholest-5-en-7-one (**104**).[48] The structures of newly synthesized compounds have been assigned on the basis of elemental analysis and spectral (IR, ^{1}H NMR, ^{13}C NMR, MS) studies. With the best of our knowledge there are no literature data available regarding the synthesis of steroidal pyranone derivatives using chitosan as a catalyst.



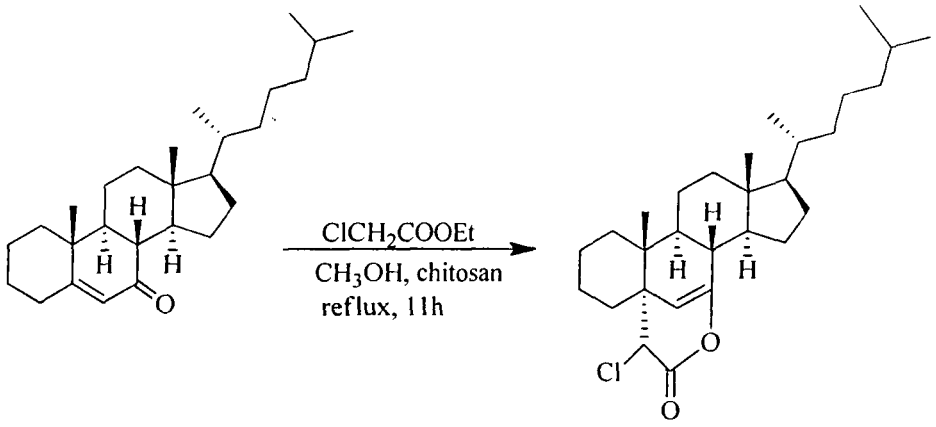
(102-104) **(105-110)**

X		X	R		R	
H	**(102)**	H	Cl	**(105)**	CH_3CO	**(108)**
OAc	**(103)**	OAc	Cl	**(106)**	CH_3CO	**(109)**
Cl	**(104)**	Cl	Cl	**(107)**	CH_3CO	**(110)**

Green synthesis and biological evaluation of steroidal 2H-pyrans as anticancer and antioxidant agents. Shamsuzzaman, **Ashraf Mashrai**, H. Khanam, M. Asif, A. Ali, A. Sherwani and M. Owais.
Journal of King Saud University-Science **2013** (in press);
http://dx.doi.org/10.1016/j.jksus.2013.10.001

Reaction of cholest-5-en-7-one (102) with ethyl chloroacetate: 3'-Chloro-3',6'-dihydro-5α- cholest-6-eno-[5, 7- d e]-2H-pyran-2-one (105):

Steroidal α,β-unsaturated ketone **102** in methanol was allowed to reflux with ethyl chloroacetate in the presence of chitosan (20 mol%) for 11 h, after usual work up it provided a single product **105**, m.p. 124-126 °C.



(102) **(105)**

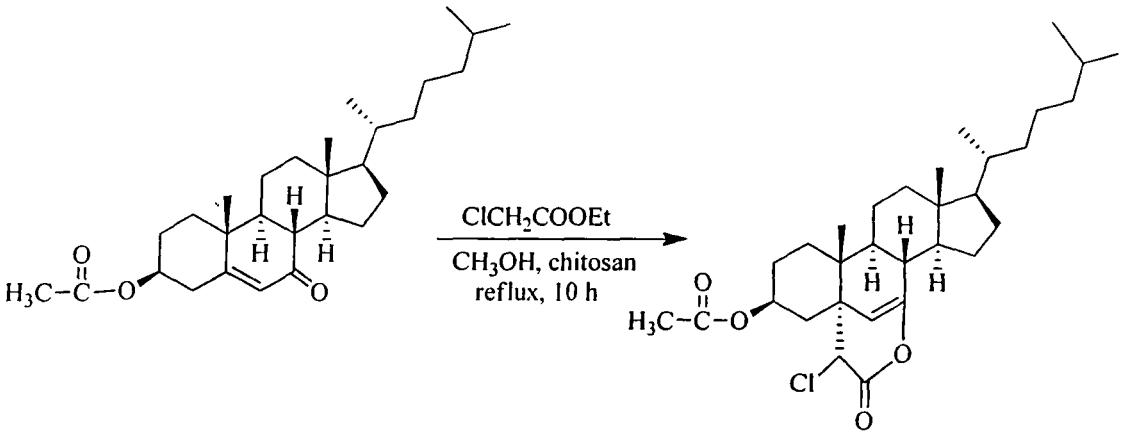
Characterization of compound 105 as 3'-chloro-3',6'-dihydro-5α-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one:

The elemental analysis of the compound **105** corresponded to the molecular formula $C_{29}H_{45}ClO_2$ (Beilstein positive). The IR data provided evidence for the formation of the expected product. The compound showed intense band in the region of 1730 cm^{-1} due to O*C*OCHCl stretching vibrations. In addition, other important absorption bands at 1614, 1145 and 751cm^{-1} were attributed to C=C, C-O and C-Cl stretchings, respectively. Further evidence for the formation of compound **105** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited a one-proton singlet at δ 5.1 for C_6-H and another one proton singlet at 4.3 for C_3'-H. Angular and side-chain methyl protons were observed at δ 1.19 (C10-CH_3), 0.75 (C13-CH_3), 0.91 and 0.85 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 162 for *C*=O, 150 for C_6, 122 for C_7 and 66 for C_3', in addition to the signals of cholestane series. The mass spectrum was also in good agreement with its molecular formula which exhibited strong molecular ion peak at *m/z* 460/462.

On account of the above descriptive discussion, the compound **105** can be suitably characterized as *3'-chloro-3',6'-dihydro-5α-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one.*

3',6'-dihydro-3β-acetoxy-5α-cholest-6-eno-[5, 7- d e]-2H-pyran-2 one (106):

Steroidal α,β-unsaturated ketone **103** in MeOH was allowed to reflux with ethyl chloroacetate in the presence of chitosan (20 mol%) for 10 h, after usual work up, a single product (**106**), m.p. 123-125 °C, was obtained.



(103) **(106)**

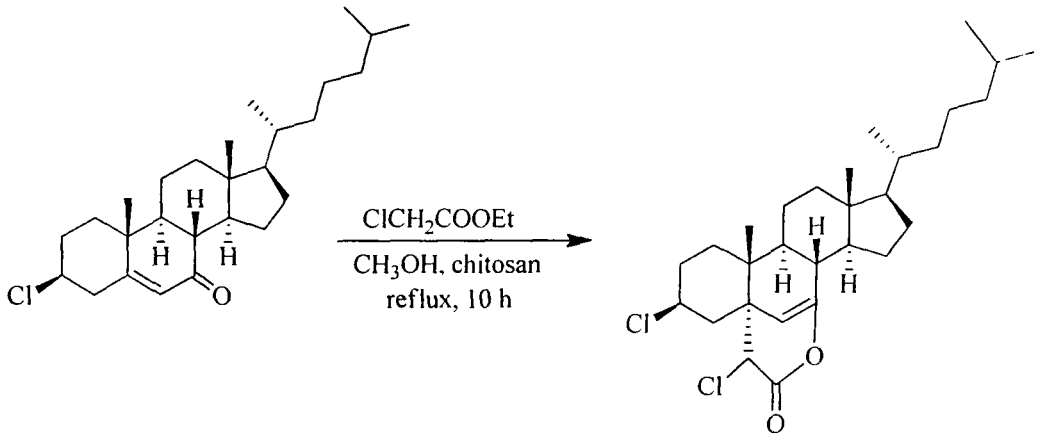
***Characterization of compound 106 as 3'-chloro-3',6'-dihydro-3β-acetoxy-5α-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one*:**

The elemental analysis of the compound **106** corresponded to the molecular formula $C_{31}H_{47}ClO_4$ (Beilstein positive). The IR data suggested the formation of the expected product. The compound showed intense band in the region of 1735 cm^{-1} due to *OCO*CHCl stretching vibration. In addition, other important absorption bands at 1615, 1142 and 754 cm^{-1} were attributed to C=C, C-O and C-Cl, respectively. Further evidence for the formation of compound **106** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited a one proton singlet at δ 5.3 for C_6-H and one proton singlet at 4.4 for C_3'-H. A broad multiplet integrating for one proton at δ 4.6 was assigned to C3-αH (axial, $W\frac{1}{2}$ = 12 Hz) and a sharp singlet for three acetoxy group protons appeared at 2.03. Angular and side-chain methyl protons were observed at δ 1.18 (C10-CH_3), 0.70 (C13-CH_3), 0.92 and 0.85 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 170 for C_3, 161 for *C*=O, 150 for C_6, 120 for C_7 and 68 for C_3', in addition to the signals of cholestane series. The mass spectrum was also in agreement with its molecular formula which exhibited a prominent molecular ion peak at *m/z* 518/520.

On account of the above evidences, the compound **106** can be suitably characterized as *3'-chloro-3',6'-dihydro-3β-acetoxy-5α-cholest-6-eno-[5, 7- d e]-2H-pyran-2 one.*

Reaction of 3β-chlorocholest-5-en-7-one (104) with ethyl chloroacetate: 3β,3'-Dichloro-3',6'-dihydro-5α cholest-6-eno-[5, 7- d e]-2H-pyran-2-one (107)

Steroidal α,β-unsaturated ketone **104** in MeOH was allowed to reflux with ethyl chloroacetate in the presence of chitosan (20 mol%) for 10 h, after usua work up and recrystallization provided a single product **107**, m.p. 122-124 °C, was obtained.



(104) **(107)**

Characterization of compound 107 as 3β,3'-dichloro-3',6'-dihydro-5α-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one:

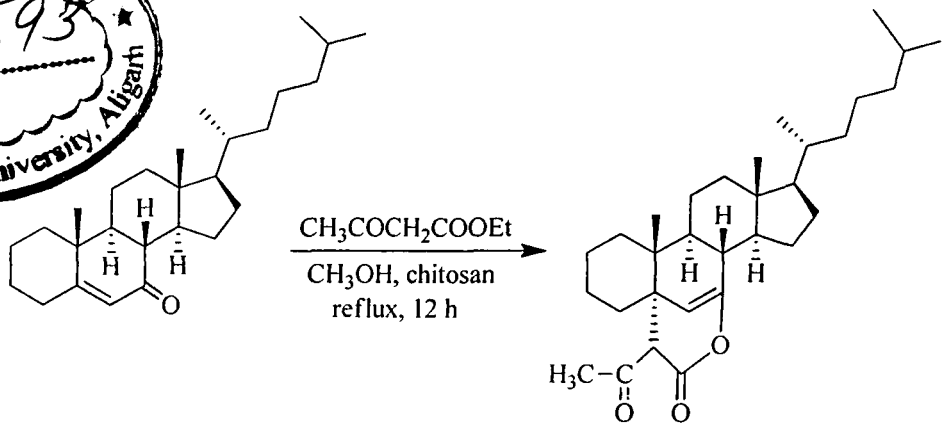
The compound **107** was correctly analyzed for $C_{29}H_{44}Cl_2O_2$ (Beilstein positive). The IR data provided evidence for the formation of the expected product. The compound showed intense band in the region of 1732 cm^{-1} due to O*CO*CHCl moiety. In addition, other important absorption bands at 1612, 1140 and 752 cm^{-1} were attributed to C=C, C-O and C-Cl stretchings, respectively. Further evidence for the formation of compound **107** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited a one proton singlet at δ 5.1 for C_6-H and another one proton singlet at 4.2 for C_3'-H. A one-proton broad multiplet centered at δ 3.8 was assigned to C3-αH (axial, $W\,½$ = 12 Hz). Angular and side-chain methyl protons were observed at δ 1.19 (C10-CH_3), 0.75 (C13-CH_3), 0.92 and 0.86 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 162 for

C=O, 150 for C_6, 122 for C_7, 67 for C_3' and 59 for C_3, in addition to the signals of cholestane series. The mass spectrum was also in agreement with its molecular formula which exhibited a strong molecular ion peak at *m/z* 494/496.

On account of the above descriptive discussion, the compound **107** can be suitably characterized as *3β,3'-dichloro-3',6'-dihydro-5α-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one.*

Reaction of cholest-5-en-7-one (102) with ethyl acetoacetate: 3'-Acetyl-3',6'-dihydro-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one (108):

Steroidal α,β-unsaturated ketone **102** in methanol was refluxed with ethyl acetoacetate in the presence of chitosan (20 mol%) for 12 h, after usual work up and recrystallization a single product **108**, m.p. 170-172 °C, was obtained.



(102) **(108)**

Characterization of compound 108 as 3'-acetyl-3',6'-dihydro-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one:

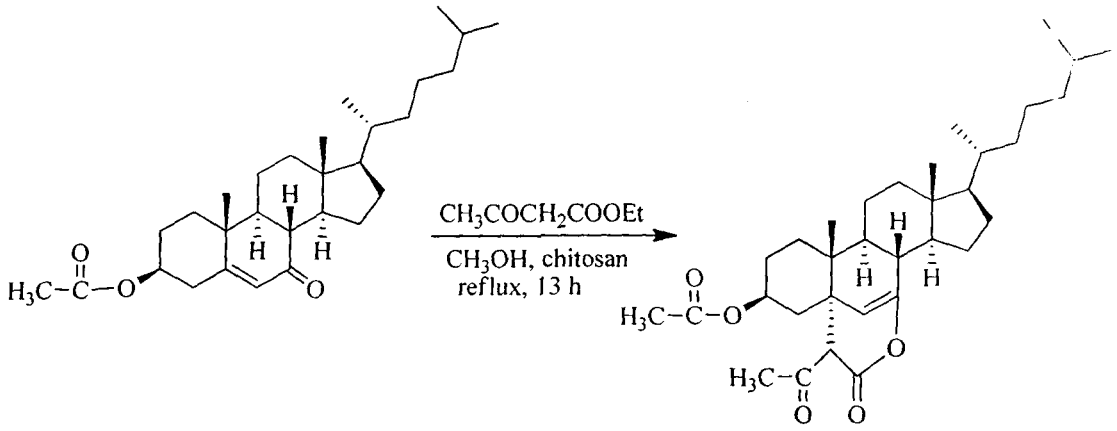
The elemental analysis of the compound **108** corresponded to the molecular formula $C_{31}H_{48}O_3$. The IR data provided evidence for the formation of the expected product. The compound showed intense band in the region of 1720 due to CH*CO*CH_3, while 1677 due to O*CO*CH, stretching vibrations. In addition, other important absorption bands at 1621 and 1246 cm^{-1} were attributed to C=C and C-O respectively. Further evidence for the formation of compound **108** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited a one-proton singlet at δ 5.31 for C_6-H and another singlet for one proton at 3.31 for C_3'-H. Angular and side-chain methyl protons were observed at δ 1.18 (C10-CH_3), 0.70 (C13-CH_3), 0.92 and 0.85 for other methyl protons. ^{13}C NMR spectrum of the

compound also supported the proposed structure and displayed characteristic signals at δ 165 for CH*C*OCH$_3$, 160 for O*C*OCH, 158 for C_7, 116 for C_6, and 65 for C'_3,in addition to the signals of cholestane series. The mass spectrum was also in good agreement with its molecular formula which exhibited strong molecular ion peak at *m/z* 468.

On account of the above evidences, the compound **108** can be suitably characterized as *3'-acetyl-3',6'-dihydro-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one*

Reaction of 3β-acetoxycholest-5-en-7-one (103) with ethyl acetoacetate: 3β-Acetoxy-3'-acetyl-3',6'dihydro-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one (109):

Steroidal *α,β*-unsaturated ketone **103** in methanol was allowed to reflux with ethyl acetoacetate in the presence of chitosan (20 mol%) for 13 h. Usual work up and recrystallization provided a single product **109**, m.p. 176-178 °C.



(103) **(109)**

Characterization of compound (109) as 3β-acetoxy-3'-acetyl-3',6'-dihydro-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one:

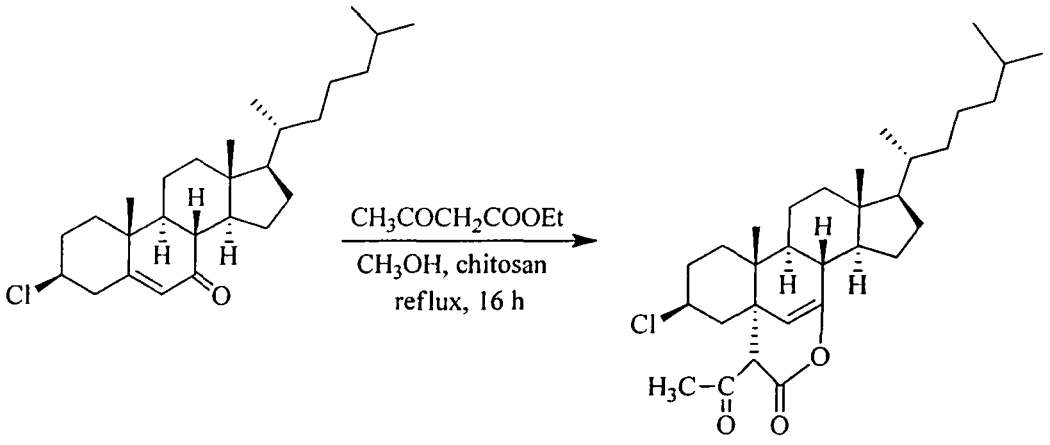
The elemental analysis of the compound **109** corresponded to the molecular formula $C_{33}H_{50}O_5$. The IR data provided evidence for the formation of the expected product. The compound showed intense band in the region of 1715 due to CH*C*OCH$_3$, while 1673 due to O*C*OCH stretching vibrations. In addition, other important absorption bands at 1622 and1245 cm^{-1} were attributed to C=C and C-O, respectively. Further evidence for the formation of compound **109** was well supported by its ^{1}H NMR and ^{13}C NMR spectra. The ^{1}H NMR spectrum of the compound exhibited a one- proton singlet at δ 5.21 for C_6-H and another one-proton broad multiplet centered at δ 4.7 was assigned to C3-αH (axial, W ½ = 16 Hz). A singlet for one proton at δ 3.21 was assigned to C_3'-H and a sharp singlet for three acetoxy group protons

appeared at 2.03. Angular and side-chain methyl protons were observed at δ 1.18 (C10-CH_3), 0.70 (C13-CH_3), 0.92 and 0.85 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 168 for CH*C*OCH_3, 163 for O*C*OCH, 152 for C_7, 112 for C_6, 72 for C_3 and 64 for C'_3, in addition to the signals of cholestane series. The mass spectrum was also in good agreement with its molecular formula which exhibited prominent molecular ion peak at *m/z* 526.

On account of the above evidences, the compound **109** can be suitably characterized as *3β-acetoxy-3'-acetyl-3',6'-dihydro-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one*

Reaction of 3β-chlorocholest-5-en-7-one (104) ethyl acetoacetate: 3β-Chloro-3'-acetyl-3',6'-dihydro-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one (110):

Steroidal ketone **104** in methanol was allowed to reflux with ethyl acetoacetate in the presence of chitosan (20 mol%) for 16 h, after usual work up and recrystallization a single product **110**, m.p. 165-167 °C, was obtained.



(104) **(110)**

Characterization of compound 110 as 3β-chloro-3'-acetyl-3',6'-dihydro-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one:

The elemental analysis of the compound corresponded to the molecular formula $C_{31}H_{47}ClO_3$ (Beilstein positive). The IR data provided evidence for the formation of the expected product. The compound showed intense band in the region of 1718 due to CH*C*OCH_3, while 1675 due to O*C*OCH stretching vibrations. In addition, other important absorption bands at 1620, 1243 and 745 cm^{-1} were attributed to C=C, C-O and C-Cl, respectively. Further evidence for the formation of compound **110** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum

of the compound exhibited a one-proton singlet at δ 5.40 for C_6-H and another one-proton broad multiplet centered at δ 3.81 was assigned to C3-αH (axial, $W_{1/2}$ = 17 Hz). Another singlet for one proton at δ 3.1 was assigned to C_3'-H. Angular and side-chain methyl protons were observed at δ 1.18 (C10-CH_3), 0.70 (C13-CH_3), 0.92 and 0.85 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 167.1 for CH*C*OCH_3, 161.3 for O*C*OCH, 159 for C_7, 114 for C_6, 64 for C'_3 and 52 for C_3, in addition to the signals of cholestane series. The mass spectrum was also in good agreement with its molecular formula which exhibited strong molecular ion peak at *m/z* 502/ 504.

On account of the above evidences, the compound **110** can be suitably characterized as *3β-chloro-3'-acetyl-3',6'-dihydro-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one*

The catalytic performance of chitosan in the synthesis of steroidal pyranone

The catalytic system is influenced by various reaction parameters, such as amount of the catalyst employed, effect of catalyst and solvent system. Therefore, in our initial investigation to establish the optimum reaction conditions 3β-acetoxy-cholest-5-en-7-one and ethyl acetoacetate in methanol were selected as model substrates. We have applied a wide range of basic catalysts such as piperidine, NaOMe, pyridine, triethylamine and chitosan to improve the yield for the specific synthesis of derivatives. As shown in **Table 1**, chitosan is superior to other catalysts in terms of reaction time and yield.

Table 1. Comparison of the efficiency of chitosan on the model reaction.

Entry	Catalysts	Time (h)	Yield (%)[a]
1	Piperidine	20	60
2	NaOMe	32	43
3	Pyridine	25	50
4	Triethylamine	36	34
5	Chitosan	16	73

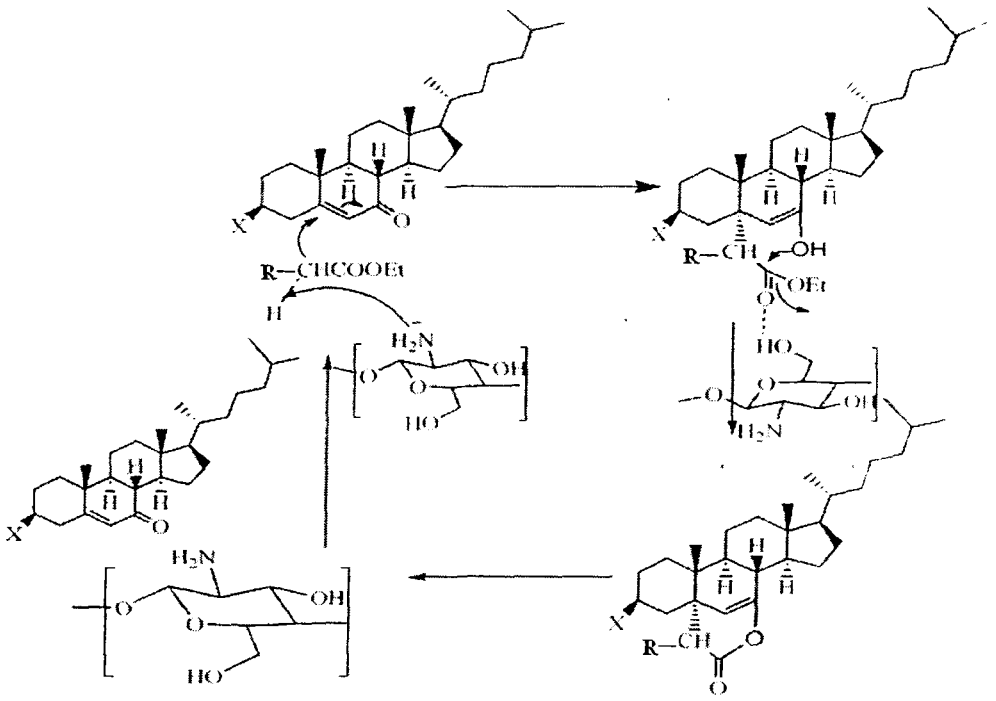
[a] Yields are related to isolated pure products.

We screened the reaction in various organic solvents in order to optimize the solvent using chitosan as catalyst. It is noteworthy to mention that the polar solvents (methanol and ethanol) afforded better yield than the non-polar ones and the best result was obtained in methanol (**Table 2**). We then tried to optimize the catalyst load for the formation of steroidal pyranone and we found that 20 mol% of the catalyst was sufficient to get optimum yield in less reaction time. Use of more than 20 mol% of the catalyst does not have any profound effect on the reaction rate as well as the yield.

Table 2. Effect of various solvents on the model reaction.

Entry	Solvent	Time(h)	Yield (%)
1	Acetonitrile	26	30
2	Methanol	16	73
3	DMSO	22	40
4	Toluene	34	22
5	Ethanol	20	60

The mechanism for the formation of steroidal pyranone derivatives involves the Michael addition of active methylene to conjugated ketone at the electrophilic alkene C, in nucleophilic addition type process, which undergoes subsequent intramolecular cyclization to provide the desired product (**Scheme 1**).



Scheme 1. Proposed mechanism of formation of 5α-cholest-6-eno-[5, 7- *d e*]-2*H*-pyran-2-one derivatives catalyzed by chitosan.

Stereochemistry

The stereochemical assignation of C5-C bond has been established on the basis of mechanism as well as on ^{1}H and ^{13}C NMR spectral analysis of the products. During the course of reaction, the nucleophilic attack of the reagent at C-5 does occur preferably from less hindered (α) side because of the steric encumbrance imposed by axial (β) methyl group at C-10, resulting axial (α) position of C5-C bond and *trans* to C10-axial methyl group (A/B ring junction *trans*). The determination of ring fusion stereochemistry in angularly methylated six-membered ring compounds is not an easy task by either chemical or physical methods, NMR spectroscopy may, however, greatly aid in the solution of this problem. ^{13}C NMR values of C-19 and C-9 are strongly dependent on the ring fusion stereochemistry. The *cis* and *trans* steroids differ most significantly at C-19 and these signal will surely be of prime value to characterize the nature of the ring junction.[49] In the all-*trans* steroids, the C-19 resonance is well separated from the majority of those of the other carbons. In the compounds (**105-110**), C-19 chemical shift values were observed in the range of 16-18 ppm, which is consistent with values obtained for *trans* steroids[50] (A/B ring junction *trans*). Furthermore the half band width ($W_{1/2}$) values of C3-axial proton in the ^{1}H NMR spectra of the synthesized compounds clearly suggest that A/B ring junction is *trans*.[51]

Experimental

General

Melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were recorded on KBr pellets with Interspec 2020 FT-IR Spectrometer spectro Lab and values are given in cm^{-1}. ^1H and ^{13}C NMR spectra were run in $CDCl_3$ on a Bruker Avance II 500 NMR Spectrometer at 500 MHz and 125 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to the TMS (^1H NMR) and to the solvent signal (^{13}C NMR spectra). Mass spectra were recorded on a JEOL D-300 mass spectrometer. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapors to check the homogeneity as well as the progress of reaction. Sodium sulfate (anhydrous) was used as a drying agent.

***Cholest-5-en-7-one* (102):**

A solution of butyl chromate [*t-butyl* alcohol (60 mL), CrO_3 (20 g), acetic acid (84 mL) and acetic anhydride (10 ml)][47] was added at 0 °C to a solution of cholest-5-ene (8 g) in CCl_4 (150 mL), acetic acid (30 ml) and acetic anhydride (10 mL). The contents were refluxed for 3 h and then diluted with water. The organic layer was washed with sodium bicarbonate solution (5 %) and water and then dried over anhydrous sodium sulfate. Evaporation of the solvents under reduced pressure furnished cholest-5-en-7-one (**63**) which was recrystallized from methanol (3 1 g), m.p. 128 °C (reported, m.p. 125-129 °C).[48a]

***3β-Acetoxycholest-5-en-7-one* (103):**

A solution of butyl chromate [*t-butyl* alcohol (60 mL), CrO_3 (20 g), acetic acid (84 mL) and acetic anhydride (10 mL][47] was added at 0 °C to a solution of 3β-acetoxycholest-5-ene (8 g) in CCl_4 (150 ml), acetic acid (30 ml) and acetic anhydride (10 mL). The contents were refluxed for 3 h and then diluted with water. The organic layer was washed with sodium bicarbonate solution (5 %) and water and then dried over anhydrous sodium sulfate. Evaporation of the solvents under reduced pressure furnished 3β-acetoxycholest-5-en-7-one which was recrystallized from methanol (3.2 g), m.p. 161-163 °C (reported, m.p. 164 °C).[48b]

***3β-Chlorocholest-5-en-7-one* (104):**

A solution of butyl chromate [*t-butyl* alcohol (60 mL), CrO_3 (20 g), acetic acid (84 mL) and acetic anhydride (10 mL)][47] was added at 0 °C to a solution of 3β-chlorocholest-5-ene (8 g) in CCl_4 (150 mL), acetic acid (30 mL) and acetic anhydride (10 mL). The contents were refluxed for 3 h and then diluted with water. The organic layer was washed with sodium bicarbonate solution (5 %) and water and then dried

over anhydrous sodium sulfate. Evaporation of the solvents under reduced pressure furnished 3β-chlorocholest-5-en-7-one which was recrystallized from methanol (3.4 g), m.p. 144 °C (reported, m.p. 144-145 °C).[48c]

***General procedure for the synthesis of steroidal pyranone derivatives* (105-110):**

A mixture of steroidal α,β-unsaturated ketone (**102-104**) (1 mmol) and ethyl chloroacetate/ethyl acetoacetate (1 mmol) in methanol (30 mL) was refluxed for 10-16 h in the presence of chitosan (20 mol%). After completion of reaction, as determined by TLC, chitosan was removed by filtration. The solvents were removed under pressure and the residue was taken in diethyl ether, washed with water, dried over anhydrous sodium sulfate Recrystallization from ethanol afforded the respective products (**105-110**).

***3'-Chloro-3',6'-dihydro-5α-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one* (105)**

Yields (76%); m.p.124-126 °C; (Beilstein positive); Anal. Calcd for $C_{29}H_{45}ClO_2$: C, 75.34, H, 8.91; found; C, 75.54, H, 9.84; IR (KBr) ν cm^{-1} 1730 (OCOCCl), 1614 (C=C), 1145 (C-O), 751 (C-Cl); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.1 (s, 1H, C_6-H), 4.3 (s, 1H, C_3'-H), 1.19 (s, 3H, C10-CH_3), 0.75 (s, 3H, C13-CH_3), 0.91 and 0.85 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162, 150, 122, 66, 56, 48, 45, 44, 43, 40, 38, 37, 36, 35, 34, 31, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 16, 12; MS (EI): (*m/z*) 460/462 [$M^{+\cdot}$]

***3'-Chloro-3',6'-dihydro-3β-acetoxy-5α-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one* (106)**

Yields (80%); m.p.123-125 °C; (Beilstein positive); Anal. Calcd for $C_{31}H_{47}ClO_4$: C, 71.26, H, 8.98; found; C,71.28, H,8.99; IR (KBr) ν cm^{-1} 1735 (OCOCCl), 1712 ($OCOCH_3$), 1615 (C=C), 1142 (C-O), 754 (C-Cl); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.3 (s, 1H, C_6-H), 4.6 (m, 1H, C3α-H, *W* ½ = 12 Hz, A/B *trans*), 4.4 (s, 1H, C_3'-H), 2.21 (s, 3H, $OCOCH_3$), 1.18 (s, 3H, C10-CH_3), 0.70 (s, 3H, C13-CH_3), 0.92 and 0.85 (other methyl protons);^{13}C NMR ($CDCl_3$, 125 MHz) δ 170, 161, 152, 120, 70, 68, 57, 48, 43, 42, 40, 39, 36 , 35, 34, 33, 32, 31, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 16, 12; MS (EI): (*m/z*) 518/520 [$M^{+\cdot}$].

***3β,3'-Dichloro-3',6'-dihydro-5α-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one* (107)**

Yields (78%); mp.122-124 °C; (Beilstein positive); Anal. Calcd for $C_{29}H_{44}Cl_2O_2$: C, 70.14, H, 8.90; found; C, 70.29, H, 8.95; IR (KBr) ν cm^{-1} 1732 (OCOCCl), 1612 (C=C), 1140 (C-O), 749, 752 (2×C-Cl); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.1 (s, 1H, C_6-H), 4.2 (s,1H, C_3'-H), 3.8 (m, 1H, C3 α-H, *W* ½ = 12 Hz, A/B *trans*), 1.19 (s, 3H,

C10-CH_3), 0.75 (s, 3H, C13-CH_3), 0.92 and 0.86 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162, 150, 122, 67, 59, 53, 49, 44, 43, 42, 41, 40, 37, 36, 34, 33, 32, 31, 29, 28, 27, 26, 25, 24, 23, 22, 19, 16, 12; MS (EI): (*m/z*) 494/496 [M^+]

3'-Acetyl-3',6'-dihydro-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one **(108)**

Yield (70%); mp. 170–172 °C; Anal. Calcd for $C_{31}H_{48}O_3$: C, 79.44, H, 10.32; found; C, 79.42, H, 10.34; IR (KBr) ν cm^{-1} 1720 ($CHCOCH_3$), 1677 (OCOCH), 1621 (C=C), 1246 (C-O); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.31 (1H, s, C_6-H), 3.31 (1H, s, C'$_3$-H), 2.01 (3H, s, $CHCOCH_3$), 0.70 (3H, s, C10-CH_3), 1.18 (3H, s, C13-CH_3), 0.92 and 0.85 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165, 160, 158, 116, 65, 48, 41, 40, 39, 38, 35, 34, 32, 31, 30, 29, 28, 28, 27, 25, 25, 24, 22, 22, 22, 21, 21, 21, 20, 18, 17; MS (EI): (*m/z*) 468 [M^+].

3β-Acetoxy-3'-acetyl-3',6'-dihydro-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one **(109)**

Yield (73%); m.p. 176-178 °C; Anal. Calcd for $C_{33}H_{50}O_5$: C, 75.25, H, 9.57; found; C, 75.21, H, 9.54; IR (KBr) ν cm^{-1} 1743 (3β-AcO), 1715 ($CHCOCH_3$), 1673 ($OCOCH_3$), 1622 (C=C), 1245 (C-O); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.21 (1H, s, C_6-H), 4.80 (1H, m, C3α-H, W ½ = 16 Hz), 3.21 (1H, s, C'$_3$-H), 2.03 (3H, s, $OCOCH_3$), 2.01 (3H, s, $CHCOCH_3$), 1.18 (3H, s, C10-CH_3), 0.70 (3H, s, C13-CH_3), 0.97 and 0.82 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170, 168, 163, 157, 112, 72, 64, 48, 41, 40, 39, 38, 35, 34, 32, 31, 30, 29, 28, 28, 28, 27, 25, 24, 22, 22, 22, 21, 21, 21, 20, 18, 17; MS (EI): (*m/z*) 526 [M^+].

3β-Chloro-3'-acetyl-3',6'-dihydro-cholest-6-eno-[5, 7- d e]-2H-pyran-2-one **(110)**

Yield (71%), mp. 165-167 °C; (Beilstein positive); Anal. Calcd for $C_{31}H_{47}ClO_3$: C, 74.00, H, 9.42; found; C, 74.02, H, 9.44; IR (KBr) ν cm^{-1} 1718 ($CHCOCH_3$), 1675 (OCOCH), 1620 (C=C), 1243 (C-O), 745 (C-Cl); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.40 (1H, s, C_6-H), 3.81 (1H, m, C3α-H, W ½ = 17 Hz), 3.10 (1H, s, C'$_3$-H), 2.01 (3H, s, $CHCOCH_3$), 1.18 (3H, s, C10-CH_3), 0.70 (3H, s, C13-CH_3), 0.92 and 0.85 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167, 161, 159, 114, 64, 52, 48, 41, 40, 39, 38, 35, 34, 32, 31, 30, 29, 28, 28, 27, 25, 24, 22, 22, 22, 21, 21, 21, 20, 18, 17; MS (EI): (*m/z*) 502/ 504 [M^+].

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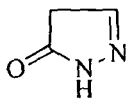
CHAPTER-3

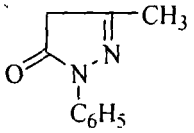
Steroidal Pyrazolones

Theoretical

The pyrazolone system (**1**), consists of a monounsaturated five membered ring with two adjacent nitrogen atoms and a carbonyl group. Knorr,[1-2] first synthesized compounds containing this system in 1883 by the reaction of ethyl acetoacetate with phenyl hydrazine, which yielded 1-phenyl-3-methyl-5-pyrazolone (**2**). Knorr[3] introduced the name to denote that the nucleus was derived from pyrrole by replacement of a carbon by nitrogen. They synthesized many members of this class and systematically investigated their properties.

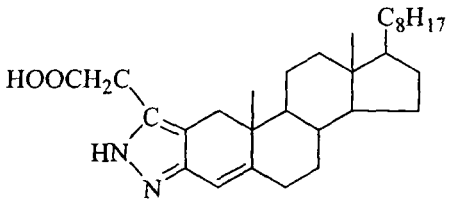


(1)



(2)

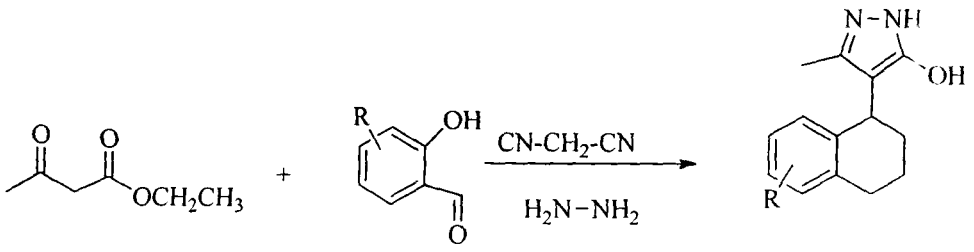
The first steroidal compound with pyrazole moiety was reported in 1938 by Ruzicka *et al.*[4] and only a single derivative, cholest-4-eno-[3,2- *c*]-pyrazole-5-carboxylic acid (**3**), was mentioned.



(3)

After a considerable span of time, much attention has been paid by a number of organic chemists towards the synthesis of several pyrazolone derivatives. The compounds containing pyrazolone ring system can be synthesized by different routes and here we have summarized only important examples, as below.

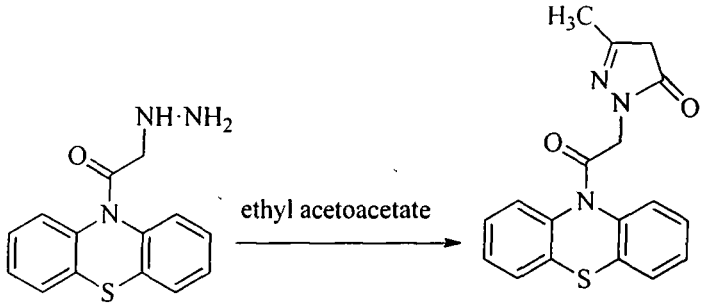
Kumaravel and Vasuki[5] synthesized 2-amino-4-(5-hydroxy-3-methyl-1*H*-pyrazol-4-yl)-4*H*-chromene-3-carbonitrile derivatives **6 (a,b)** by a four component reaction between hydrazine hydrate, ethyl acetoacetate (**4**), 2-hydroxybenzaldehydes **5 (a,b)** and malononitrile in water at ambient temperature.



(4) **(5)** **(6)**

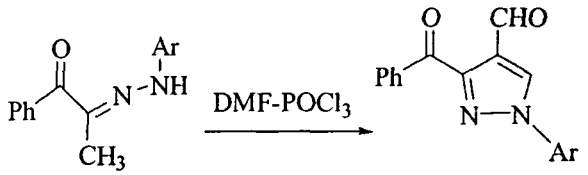
R	R
(a) 2-CH_3	(a) 2-CH_3
(b) 2-OCH_3	(b) 2-OCH_3

Bicu *et al.*[6] synthesized pyrazolones containing a phenothiazine unit (**8**), the synthesized compound also evaluated for its antiproliferative activity.



(7) (8)

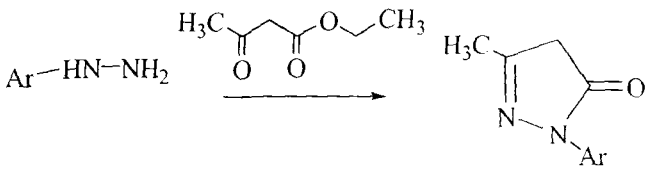
Bavatenko and coworkers[7] reported substituted pyrazoles **10 (a-d)** by cyclizing aryl hydrazones **9 (a-d)**, under Vilsmeier conditions.



(9) (10)

Ar	Ar
(a) C_6H_5	(a) C_6H_5
(b) 4-FC_6H_4	(b) 4-FC_6H_4
(c) 4-$CH_3C_6H_4$	(c) 4-$CH_3C_6H_4$
(d) 4-$CH_3OC_6H_4$	(d) 4-$CH_3OC_6H_4$

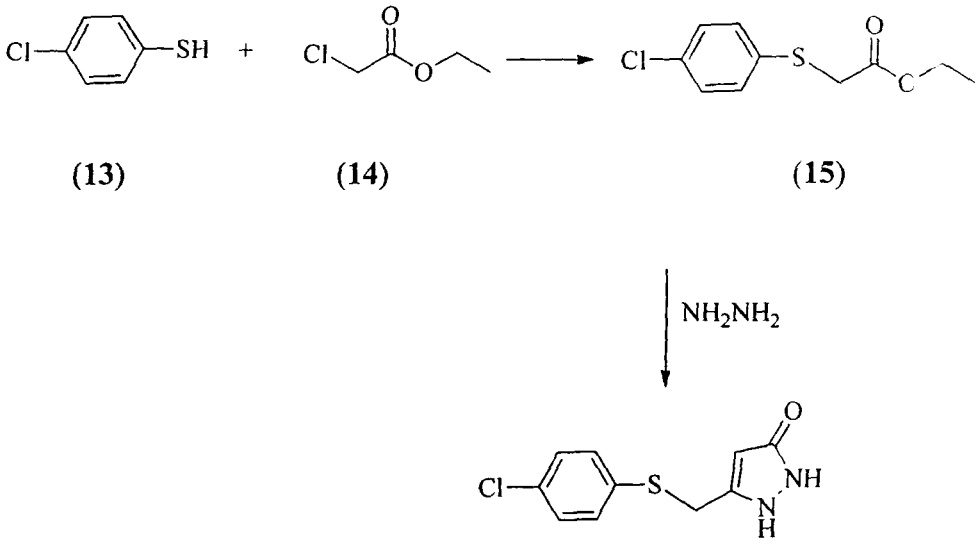
Isloor and co-workers[8] reported that the reaction of substituted hydrazines **11 (a-g)** with ethyl acetoacetate in absolute alcohol yielded corresponding substituted pyrazolones **12 (a-g)**.



(11) (12)

Ar	Ar
(a) C_6H_5	(a) C_6H_5
(b) 2,4-Dinitrophenyl	(b) 2,4-Dinitrophenyl
(c) 4-Chlorophenyl	(c) 4-Chlorophenyl
(d) 4-$OCH_3C_6H_4$	(d) 4-$OCH_3C_6H_4$
(e) Biphenyl	(e) Biphenyl
(f) 2,4-Dichlorophenyl	(f) 2,4-Dichlorophenyl
(g) 4-$SCH_3C_6H_4$	(g) 4-$SCH_3C_6H_4$

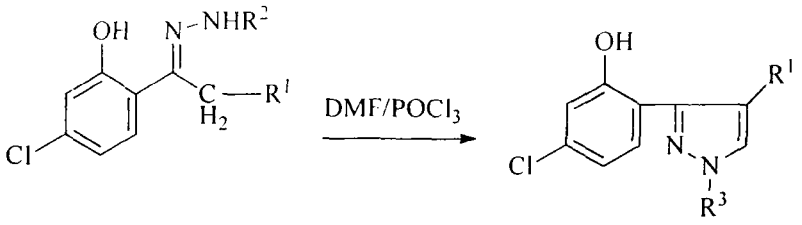
Silverman *et al.*[9] synthesized 5-((4-Chlorophenylthio)methyl)-1*H*-pyrazol-3(2*H*)-one (**16**) by the reaction of 4-Chlorothiophenol (**13**) with ethyl 4-chloroacetoacetate (**14**) following by the treatment of the product **15** with hydrazine hydrate.



(13) (14) (15)

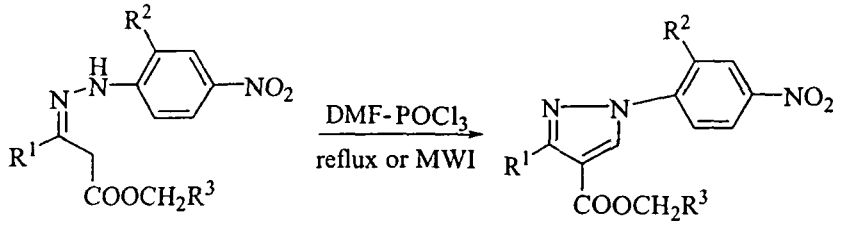
(16)

Pawar and Borse[10] reported 4-n-alkylsubstituted pyrazoles (**23-28**), from phenylhydrazones (**17-20**), semicarbazones (**21**) and azines of 2-n-acyl-5-chlorophenoles (**22**) by monoformylation and cyclization by using one mole of the Vilsmeier-Haack reagent (DMF-$POCl_3$).



	R^1	R^2		R^1	R^2
(17)	CH_3	Ph	**(23)**	CH_3	Ph
(18)	C_2H_5	Ph	**(24)**	C_2H_5	Ph
(19)	$(CH_2)_2$-CH_3	Ph	**(25)**	$(CH_2)_2$-CH_3	Ph
(20)	$(CH_2)_2$-CH_3	Ph	**(26)**	$(CH_2)_2$-CH_3	Ph
(21)	CH_3	$CONH_2$	**(27)**	CH_3	$CONH_2$
(22)	H	1-(3′-Chloro-5′-hydroxy-phenyl) propene			
(28)	CH_3	1-(3′-Cloro-5′-hydroxy-phenyl) propan-1-one			

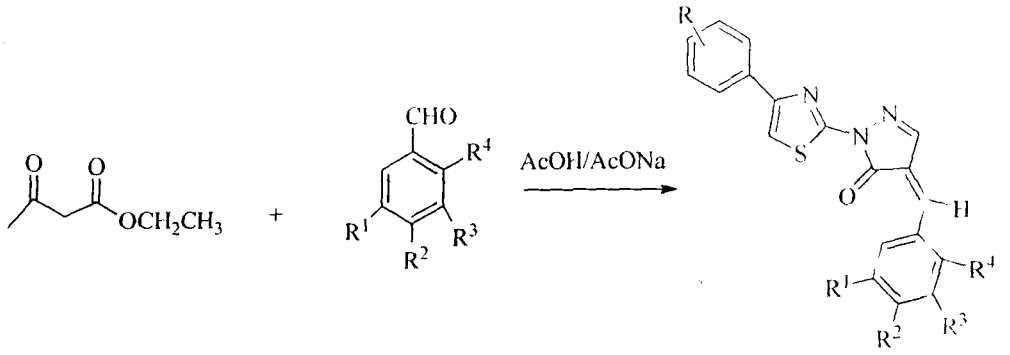
Sridhar and Perumal[11] reported the conventional and microwave synthesis of 1-*H*-pyrazole-4-carboxylic acid esters **30 (a-h)** by the reaction of hydrazones of *β*-ketoesters **29 (a-h)** with Vilsmeier reagent.



(29) **(30)**

	R^1	R^2	R^3		R^1	R^2	R^3
(a)	CH_3	H	H	**(a)**	CH_3	H	H
(b)	CH_3	NO_2	H	**(b)**	CH_3	NO_2	H
(c)	CH_3	H	CH_3	**(c)**	CH_3	H	CH_3
(d)	CH_3	NO_2	CH_3	**(d)**	CH_3	NO_2	CH_3
(e)	C_6H_5	H	CH_3	**(e)**	C_6H_5	H	CH_3
(f)	C_6H_5	NO_2	CH_3	**(f)**	C_6H_5	NO_2	CH_3
(g)	4-ClC_6H_4	H	CH_3	**(g)**	4-$C1C_6H_4$	H	CH_3
(h)	4-ClC_6H_4	NO_2	CH_3	**(h)**	4-ClC_6H_4	NO_2	CH_3

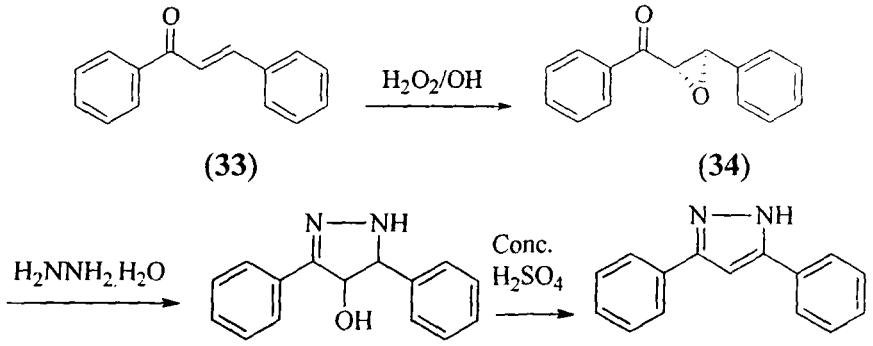
Venkata and Rao[12] synthesized 4-arylidene-3-methyl-1-(4-arylthiazol-2-yl)-1*H*-pyrazol-5(4*H*)-ones **32 (a-d)** using ethylacetoacetate and aryl aldehydes **31 (a-d)**.



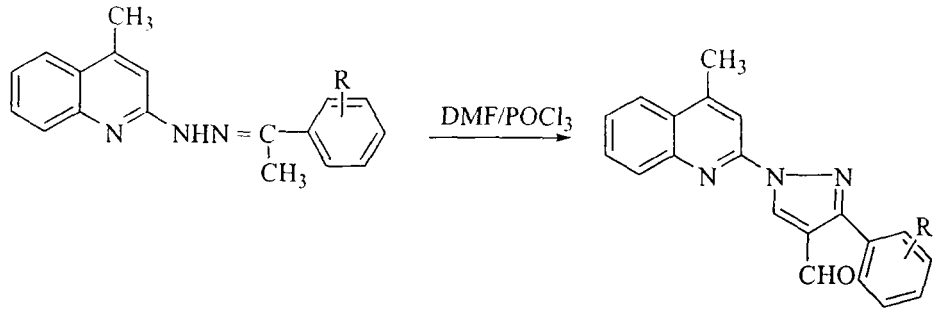
(31) **(32)**

	R^1	R^2	R^3	R^4
(a)	H	H	$N(CH_3)_2$	H
(b)	H	H	$N(CH_3)_2$	H
(c)	H	H	CH_3	H
(d)	H	H	OCH_3	H

Dhar and Bhat[13] obtained 3,5-diphenyl-pyrazoles (**36**), from chalcones (**33**), by the reaction of hydrazine hydrate on chalcone-epoxide (**34**), followed by simultaneous dehydration in presence of catalytic amount of conc. H_2SO_4 in acetic acid.



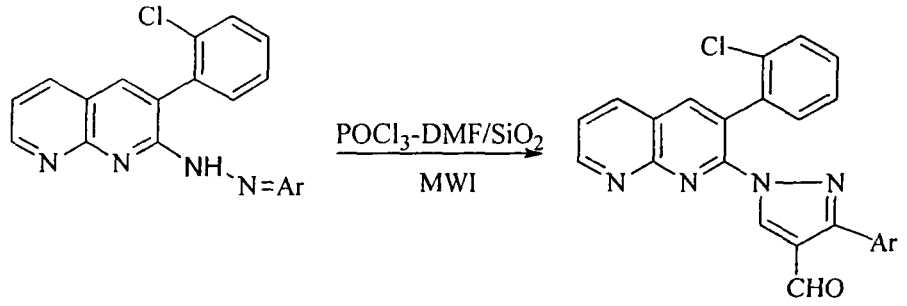
Kidwai and coworkers[14] synthesized 4-methyl-2-[3'-substituted-phenyl-4'-formylpyrazolyl] quinoline **38 (a-d)**, by the reaction of hydrazones of methyl quinoline **37 (a-d)**, with DMF and $POCl_3$ under microwave irradiation of 3-4 min.



(37) (38)

	R		R
(a)	H	(a)	H
(b)	4-Cl	(b)	4-Cl
(c)	4-CH_3	(c)	4-CH_3
(d)	4-Br	(d)	4-Br

3-Aryl-4-formyl-l[3(2-chlorophenyl)-8-naphthyridin-2-yl] pyrazoles **40 (a-d)** were yielded when hydrazones **39 (a-d)** were subjected to microwave irradiation[15] in the presence of Vilsmeier-Haack reagent.

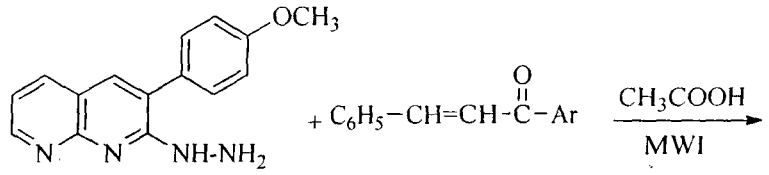


(39) (40)

	Ar		Ar
(a)	C_6H_5	(a)	C_6H_5
(b)	4-$CH_3C_6H_4$	(b)	4-$CH_3C_6H_4$
(c)	4-$CH_3OC_6H_4$	(c)	4-$CH_3OC_6H_4$
(d)	4-ClC_6H_4	(d)	4-ClC_6H_4

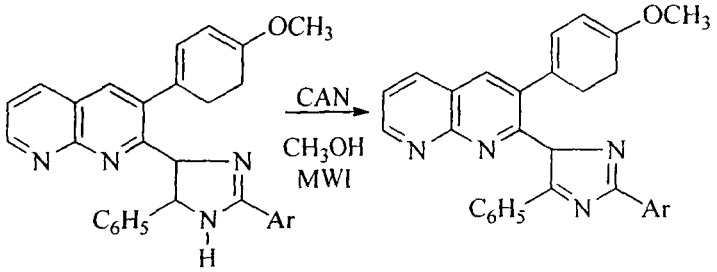
Condensation of 2-hydrazino-3-(4-methoxyphenyl)-l,8-naphthyridine **(41)** with arylidene acetophenones **42 (a,b)**, in glacial acetic acid under microwave irradiation afforded the corresponding 2-(3,5-diaryl-2-pyrazolin-l-yl)-3-(4-methoxy-phenyl)-l,8-naphthyridines **43 (a-b)**, which on oxidation with CAN in methanol under

phenyl)-1,8-naphthyridines **43** (**a-b**), which on oxidation with CAN in methanol under MWI furnished 2-(3,5-diarylpyrazol-1-yl)-3-(4-methoxyphenyl)-1,8-naphthyridines **44** (**a,b**).[16]



(41) **(42)**

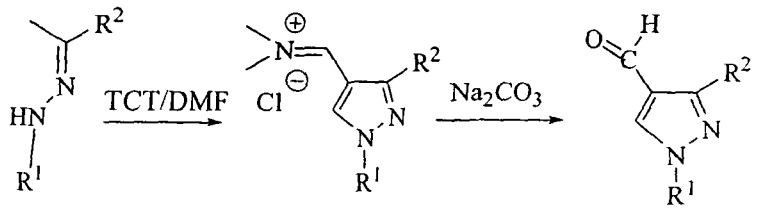
	Ar
(a)	C_6H_5
(b)	4-$CH_3OC_6H_4$



(43) **(44)**

	Ar		**Ar**
(a)	C_6H_5	**(a)**	C_6H_5
(b)	4-$CH_3OC_6H_4$	**(b)**	4-$CH_3OC_6H_4$

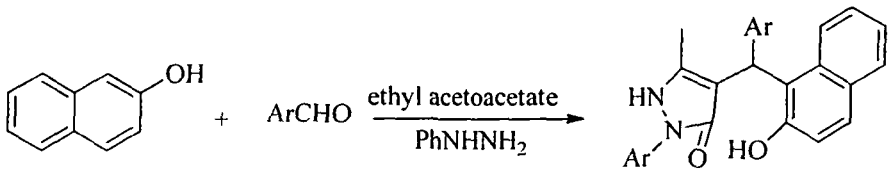
Giacomelli *et al.*[17] prepared a variety of 3-aryl-4-formyl pyrazoles **47** (**a-f**) by reaction of 1 molar equivalent of ketone hydrazones **45** (**a-f**), with 2,4,6-trichloro-[1,3,5] triazine (TCT) in DMF at room temperature and subsequent neutralization with Na_2CO_3 (15%).



(45) **(46)** **(47)**

	R^1	R^2		R^1	R^2
(a)	C_6H_5	C_6H_5	**(a)**	C_6H_5	C_6H_5
(b)	C_6H_5	$2\text{-}CH_3C_6H_4$	**(b)**	C_6H_5	$2\text{-}CH_3C_6H_4$
(c)	C_6H_5	$3\text{-}CH_3C_6H_4$	**(c)**	C_6H_5	$3\text{-}CH_3C_6H_4$
(d)	C_6H_5	$4\text{-}CH_3C_6H_4$	**(d)**	C_6H_5	$4\text{-}CH_3C_6H_4$
(e)	C_6H_5	$4\text{-}HOC_6H_4$	**(e)**	C_6H_5	$4\text{-}HOC_6H_4$
(f)	C_6H_5	$4\text{-}ClC_6H_4$	**(f)**	C_6H_5	$4\text{-}ClC_6H_4$

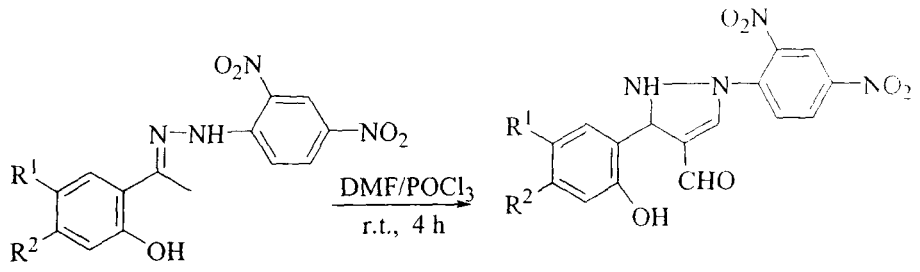
Perumal *et al.*[18] synthesized novel 2-aryl-5-methyl-2,3-dihydro-1*H*-3-pyrazolones (**50**) by reaction of phenylhydrazine, ethyl acetoacetate aromatic aldehydes **49** (**a-f**) and *2*-naphthol (**48**).



(48) **(49)** **(50)**

	R	**R**
(a)	$4\text{-}O_2NC_6H_4$	$4\text{-}O_2NC_6H_4$
(b)	$2\text{-}O_2NC_6H_4$	$2\text{-}O_2NC_6H_4$
(c)	$4\text{-}ClC_6H_4$	$4\text{-}ClC_6H_4$
(d)	C_6H_5	C_6H_5
(e)	$4\text{-}PrC_6H_4$	$4\text{-}PrC_6H_4$
(f)	$4\text{-}MeOC_6H_4$	$4\text{-}MeOC_6H_4$

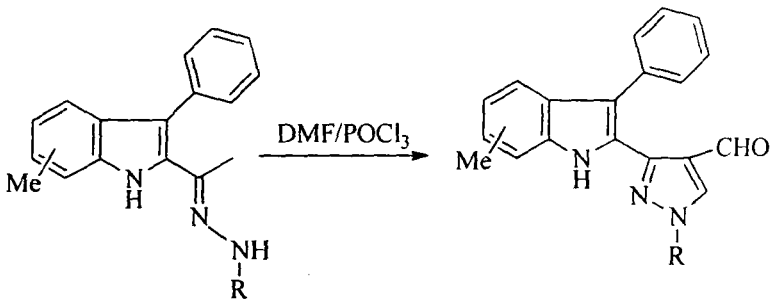
Perumal and Selvi[19] synthesized 4-ethoxy-4*H*-benzopyrano [4,3- *c*] pyrazoles **52** (**a-f**) from *o*-hydroxyacetophenone-2,4-dinitrophenylhydrazones **51** (**a-f**), under the Vilsmeier conditions.



(51) (52)

	R^1	R^2		R^1	R^2
(a)	H	H	**(a)**	H	H
(b)	H	OCH_3	**(b)**	H	OCH_3
(c)	H	CH_3	**(c)**	H	CH_3
(d)	H	Cl	**(d)**	H	Cl
(e)	H	OH	**(e)**	H	OH
(f)	OCH_3	H	**(f)**	OCH_3	H

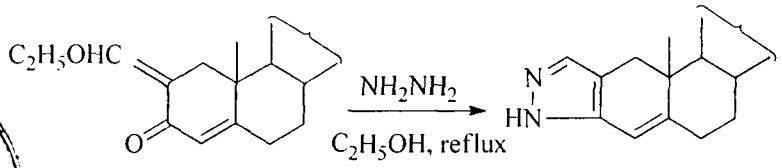
Substituted hydrazones of 2-acetyl-3-phenylindoles **53** (**a-c**) on treatment with 2 moles of Vilsmeier-Haack reagent followed by alkaline hydrolysis furnished 2-(4-formyl-3-pyrazolyl)-3-phenylindoles **54** (**a-c**).[20]



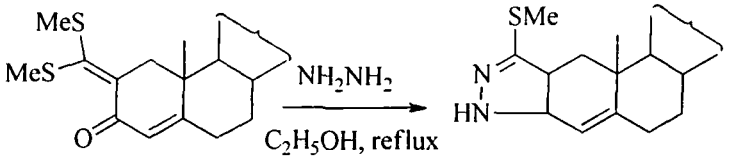
(53) (54)

R	**R**
(a) H	**(a)** H
(b) C_6H_5	**(b)** C_6H_5
(c) $SO_2C_6H_4CH_3$	**(c)** $SO_2C_6H_4CH_3$

Singh *et al.*[21] reported the synthesis of pyrazolo [3,2- *c*] cholest-4-ene (**56**) and [1'*H*]-5'-(methylthio) pyrazolo [3,2- *c*] cholest-4-ene (**58**) from 2-ethoxymethylene-cholest-4-en-3-one (**55**) and bis(methylthio) methylene cholest-4-en-3-one (**57**), respectively, by the reaction of hydrazine hydrate in ethanol under reflux conditions.

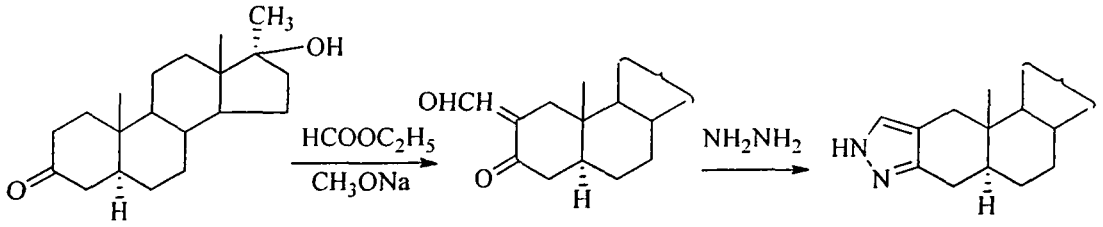


(55) (56)

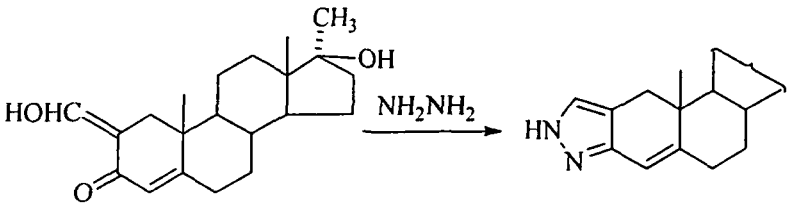


(57) (58)

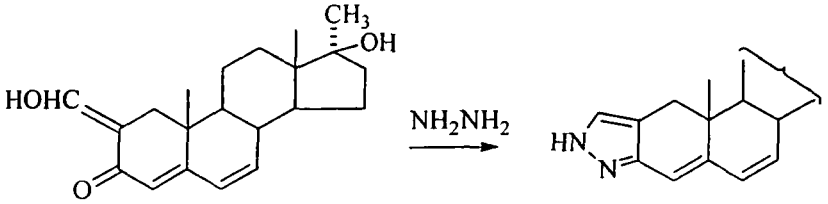
Clinton *et al.*[22] reported that treatment of 17α-methylandrostan-17β-ol-3-one (**59**) with ethyl formate and sodium methoxide gave 2-hydroxymethylene derivative (**60**) which on condensation with hydrazine gave 17β-hydroxy-17-α methylandrostano [3,2- *c*] pyrazole (**61**). Similar treatment of 2-hydroxymethylene-17α-methylandrost-4-en-17β-ol-3-one (**62**), furnished 17β-hydroxy-methylene-17α-methylandrost-4-eno [3,2- *c*] pyrazole (**63**). In the same way, the homologous 17β-hydroxy-17α-methylandrost-4,6-dieno [3,2- *c*] pyrazole (**65**), was obtained from **64**.



(59) (60) (61)

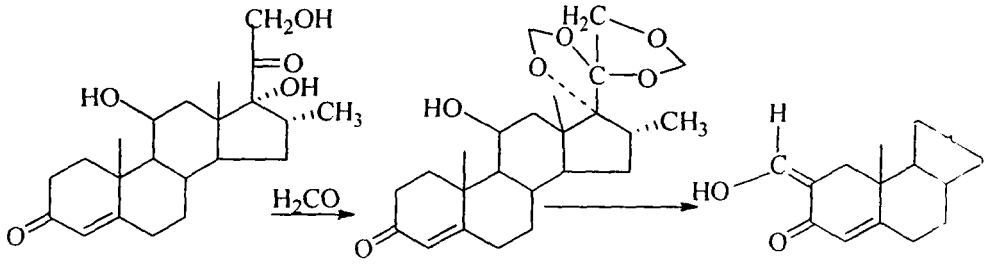


(62) (63)

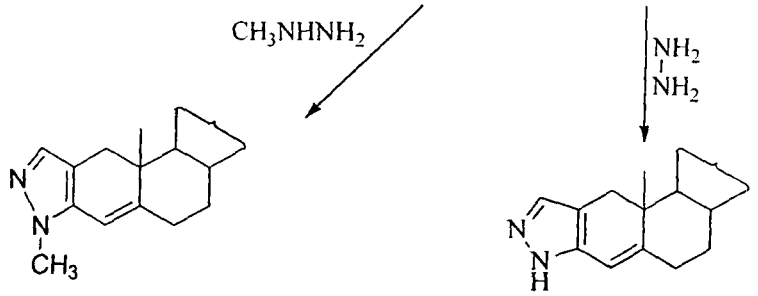


(64) (65)

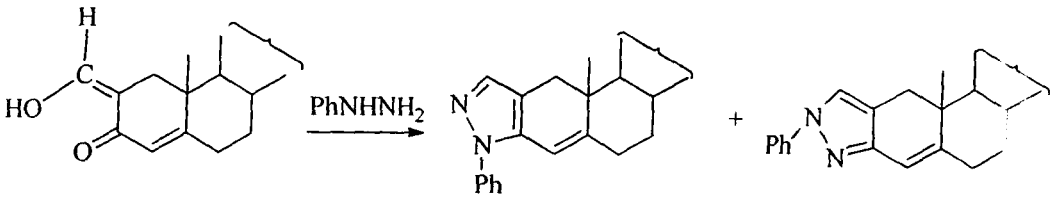
Hirschmann and coworkers[23] reported several [3,2- *c*] pyrazoles related to cortisol, 16α-methylcortisol and 4,5α-dihydrocortisol. The cortisol side chain of compound **66** was protected by formation of the bismethylenedioxy (DMD) derivative (**67**), for the synthesis of pyrazoles related to 16α-methylcortisol. The compound **67** was allowed to react with ethylformate in benzene in the presence of sodium hydride to give 2-hydroxymethylene derivative (**68**). The compound **68** was subjected to condense with hydrazine and methyl hydrazine to yield the respective [3,2- *c*] pyrazoles **69** and **70**, respectively. While treatment of compound **68** with phenyl hydrazine gave pyrazoles **71** along with minor product **72**.



(66) **(67)** **(68)**



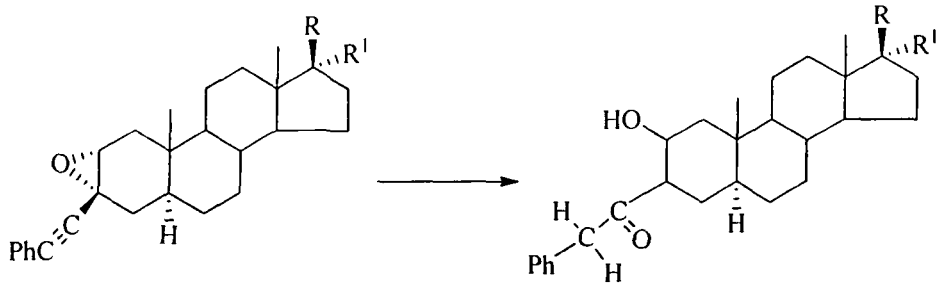
(69) **(70)**



(68) **(71)** **(72)**

(major) (minor)

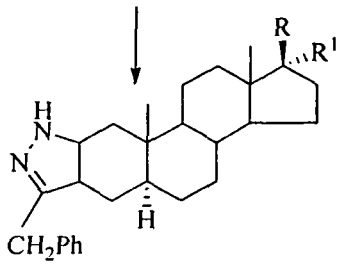
Berbalk and his coworkers[24] in 1982 reported that the epoxyandrostane **73 (a,b)** underwent formolysis to give phenyl acetylandrostane **74 (a,b)** which on further cyclocondensation with hydrazine hydrate afforded androstano-pyrazoles **75 (a,b)**.



(73) **(74)**

	R	R¹
(a)	OAc	H
(b)	OH	H

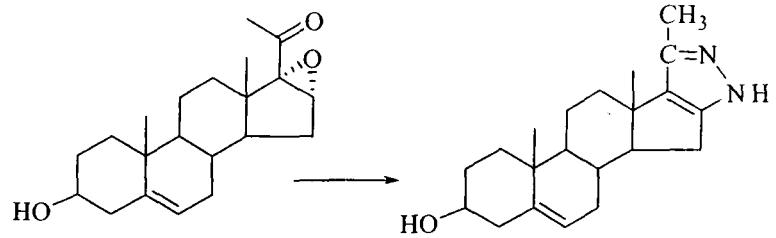
	R	R¹
(a)	OAc	H
(b)	OH	H



(75)

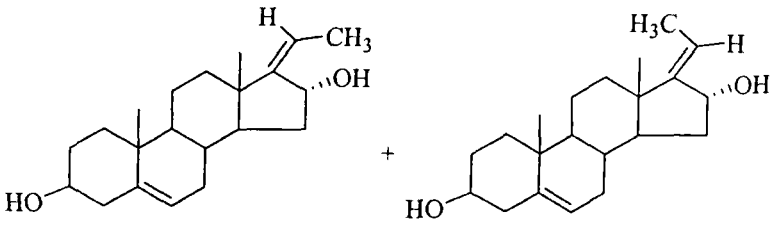
	R	R¹
(a)	OAc	H
(b)	OH	H

Bonn and Dodson[25] carried out the hydrazine reduction of 16α,17α-epoxy-pregnenolone (**76**) to obtain 3β-hydroxyandrost-5-ena[16,17- *c*]-5'-methylpyrazole (**77**) along with the two isomeric allylic alcohols, 5,17 [20]-(*cis*)-pregnadiene-3β,16α-diol (**78**) and 5,17 [20]-(*trans*)-pregnadiene-3β,16α-diol (**79**).



(76) **(77)**

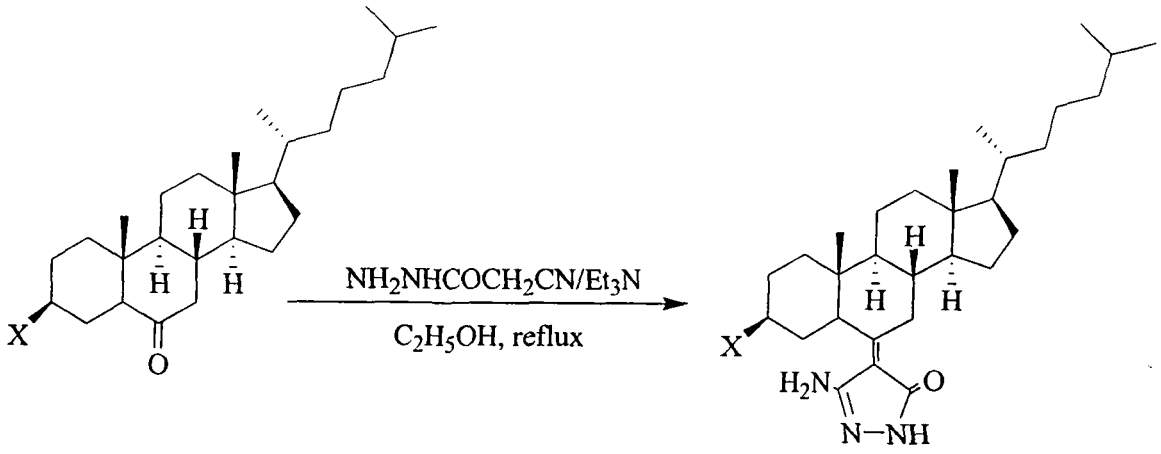
+



(78) **(79)**

Discussion

As a part of our continuing effort towards the synthesis of modified steroids, which are expected to be biologically active, the fusion of heterocycles to steroids often led to a change in physiological activities or appearance of new interesting biological behavior. Thus, several steroidal heterocycles have been obtained exhibiting activity like potential inhibitors of cytochrome P450 enzyme aromatage,[26,27] with their subsequent clinical application in the treatment of estrogen dependent breast cancer. Pyrazolones constitute a class of compounds associated with wide spread popularity in the field of medicine,[28-30] and agrochemistry,[12] as evident from number of reports covering their preparations and uses. Many of these were found to possess antimicrobial, anti-inflammatory, hypotensive, hypocholesterolemic and diuretic activities.[31-39] The therapeutic importance of modified steroids[20] encouraged us to synthesize steroidal pyrazolones. The substrates selected for initial studies include 5α-cholestan-6-one (**90**)[40] 3β-acetoxy-5α-cholestan-6-one (**91**)[41] and 3β-chloro-5α-cholestan-6-one (**92**).[42] The products obtained have been characterized on the basis of their elemental analysis and spectral (IR, ^{1}H NMR, ^{13}C NMR and MS) studies.



(90-92)

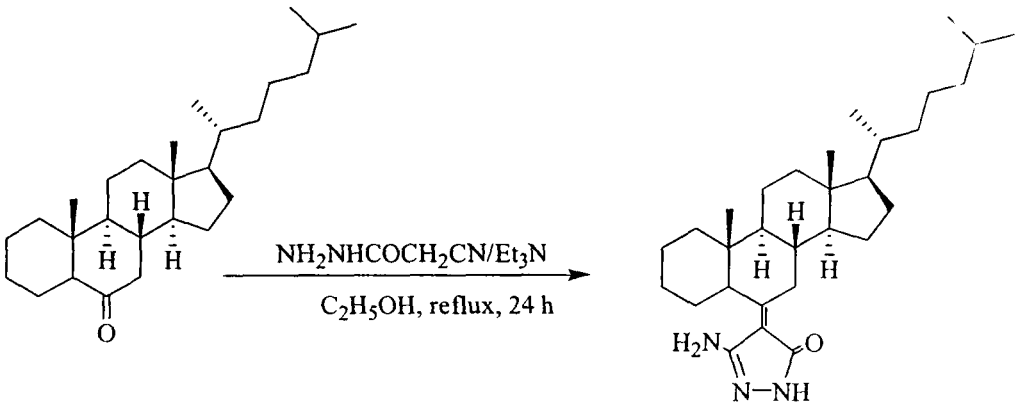
(93-95)

X	X
(80) H	(83) H
(81) OAc	(84) OAc
(82) Cl	(85) Cl

Synthesis, evaluation and docking studies on steroidal pyrazolones as anticancer and antimicrobial agents. Shamsuzzaman, **Ashraf Mashrai**, A. Ahmad, A. M. Dar, H. Khanam, M. Danishuddin and A. U. Khan
Med. Chem. Res. **(2014) 23:348-362**

Reaction of 5α-cholestan-6-one (80) with cyanoacetohydrazide: 6-(5'-Amino-3'-oxo-dihydro -4H-pyrazol-4'-ylidine)-5α-cholestane (83):

To a solution of steroidal ketone **80** (1 mmol) in ethanol (15 mL), cyanoacetohydrazide (1 mmol) and few drops of triethyl amine were added. The reaction mixture was refluxed for 24 h. After completion of the reaction it was usually worked up. Recrystallization from methanol provided a single product **83**, m.p. 134-135 °C.



(80) **(83)**

Characterization of compound 83 as 6-(5'-amino-3'-oxo-dihydro-4H-pyrazol-4'-ylidine)-5α-cholestane

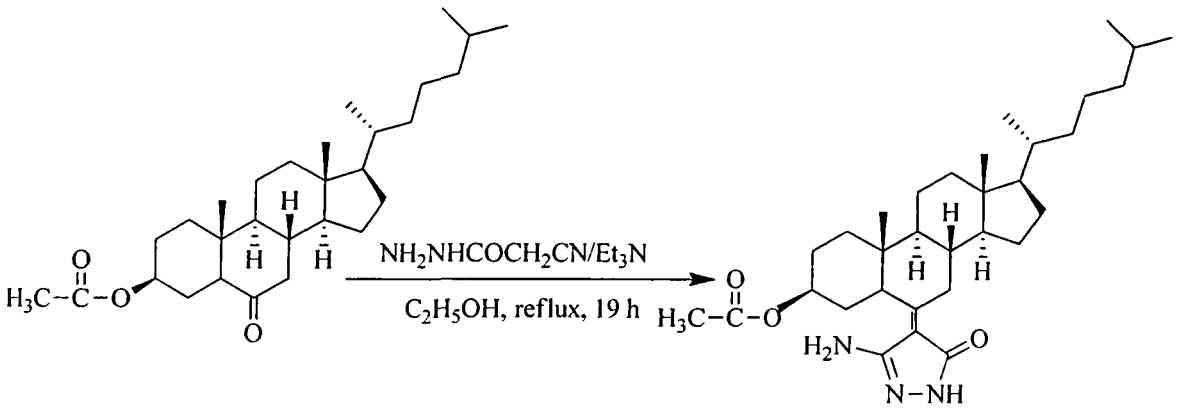
The elemental analysis of compound **83** corresponded to the molecular formula $C_{30}H_{49}N_3O$. The IR data provided evidence for the formation of the expected product. The compound showed intense bands in the region of 3385 and 3230 cm^{-1} due to NH and NH_2 stretching vibrations, respectively. In addition, other important absorption bands at 1691, 1657 and 1622 cm^{-1} were attributed to C=O, C=N and C=C stretchings, respectively. Further evidence for the formation of compound **83** was well supported by its ^1H NMR and ^{13}C NMR spectra. Its ^1H NMR spectrum exhibited one-proton singlet at δ 8.9 for NH (exchangeable with D_2O) and another two-proton singlet at 2.6 for NH_2 (exchangeable with D_2O). Angular and side-chain methyl protons were observed at δ 1.19 (C10-CH_3), 0.75 (C13-CH_3), 0.96 and 0.83 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 173 for *C*O, 150 for *C*=N, 140 for C_6 and 119 for C'_4, in addition to the known signals of cholestane series. The mass

spectrum was also in good agreement with its molecular formula which exhibited a prominent molecular ion peak at *m/z* 467.

On account of the above descriptive discussion, the compound **83** can be suitably characterized as *6-(5'-amino-3'-oxo-dihydro-4H-pyrazol-4'-ylidine)-5α-cholestane.*

Reaction of 3β-acetoxy-5α-cholestan-6-one (81) with cyanoacetohydrazide: 3β-Acetoxy-6-(5'-amino-3'-oxo-dihydro-4H-pyrazol-4'-ylidine)-5α-cholestane (84):

To a solution of steroidal ketone **81** (1 mmol) in ethanol (15 mL), equimolar quantity of cyanoacetohydrazide and few drops of triethyl amine were added then the reaction mixture was refluxed for 19 h. After completion of the reaction and usual work up, a single product **84**, m.p. 159-160 °C, was obtained.



(81) **(84)**

Characterization of compound 84 as 3β-acetoxy-6-(5'-amino-3'-oxo-dihydro-4H-pyrazol-4'-ylidine)-5α-cholestane

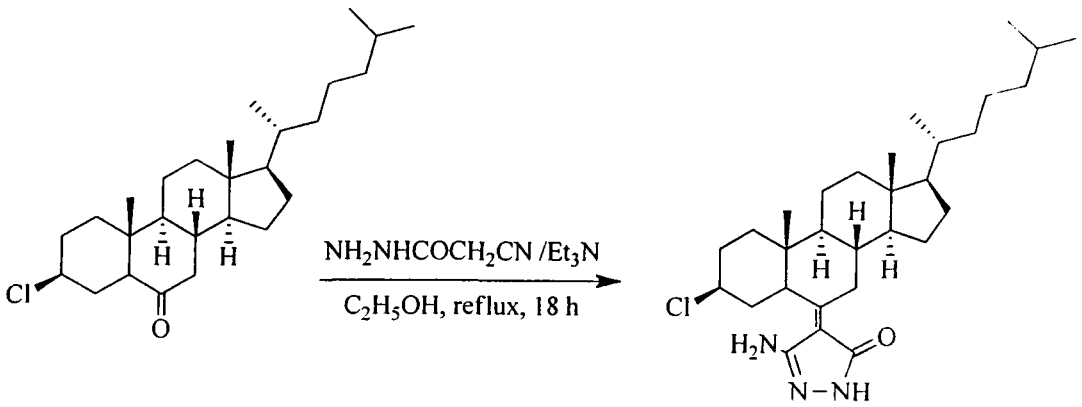
The compound **84** was correctly analyzed for formula $C_{32}H_{51}N_3O_3$. The IR analysis of the compound showed intense bands in the region of 3390 and 3210 cm^{-1} due to NH and NH_2 stretching vibrations, respectively. In addition, other important absorption bands at 1763, 1689, 1655, 1628 and 1240 cm^{-1} were attributed to C=O (ester), C=O, C=N, C=C and C-O stretchings, respectively. Further evidence for the formation of structure **84** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited one-proton singlet at δ 8.6 for NH (exchangeable with D_2O) while a two proton singlet at 2.5 was assigned to NH_2 (exchangeable with D_2O). A broad multiplet centered at δ 4.7 was assigned to C3-αH

(axial, $W½$ = 15 Hz) and a three-proton sharp singlet at 2.03 was attributed to $OCOCH_3$. Angular and side-chain methyl protons were observed at 1.19 (C10-CH_3), 0.75 (C13-CH_3), 0.97 and 0.83 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 171 for *C*O, 167 for *C*ONH and 154 for *C*=N, 145 for C_6 and 118 for C'_4, in addition to the expected signals of cholestane series. The mass spectrum was also in agreement with its molecular formula which exhibited a strong molecular ion peak at *m/z* 525.

On account of the above discussion, the compound **84** can be suitably characterized as *3β-acetoxy-6-(5'-amino-3'-oxo-dihydro-4H-pyrazol-4'-ylidine)-5α-cholestane.*

Reaction of 3β-chloro-5α-cholestan-6-one (82) with cyanoacetohydrazide: 3β-Chloro-6-(5'-amino-3'-oxo-dihydro-4H-pyrazol-4'-ylidine)-5α-cholestane (85):

To a solution of steroidal ketone **82** (1 mmol) in ethanol (15 mL), cyanoacetohydrazide (1 mmol) few drops of triethyl amine were added. The reaction mixture was refluxed for 18 h. After completion of reaction, Usual work up and recrystallization, it provided a single product **85**, m.p. 147-148 °C.



(82) **(85)**

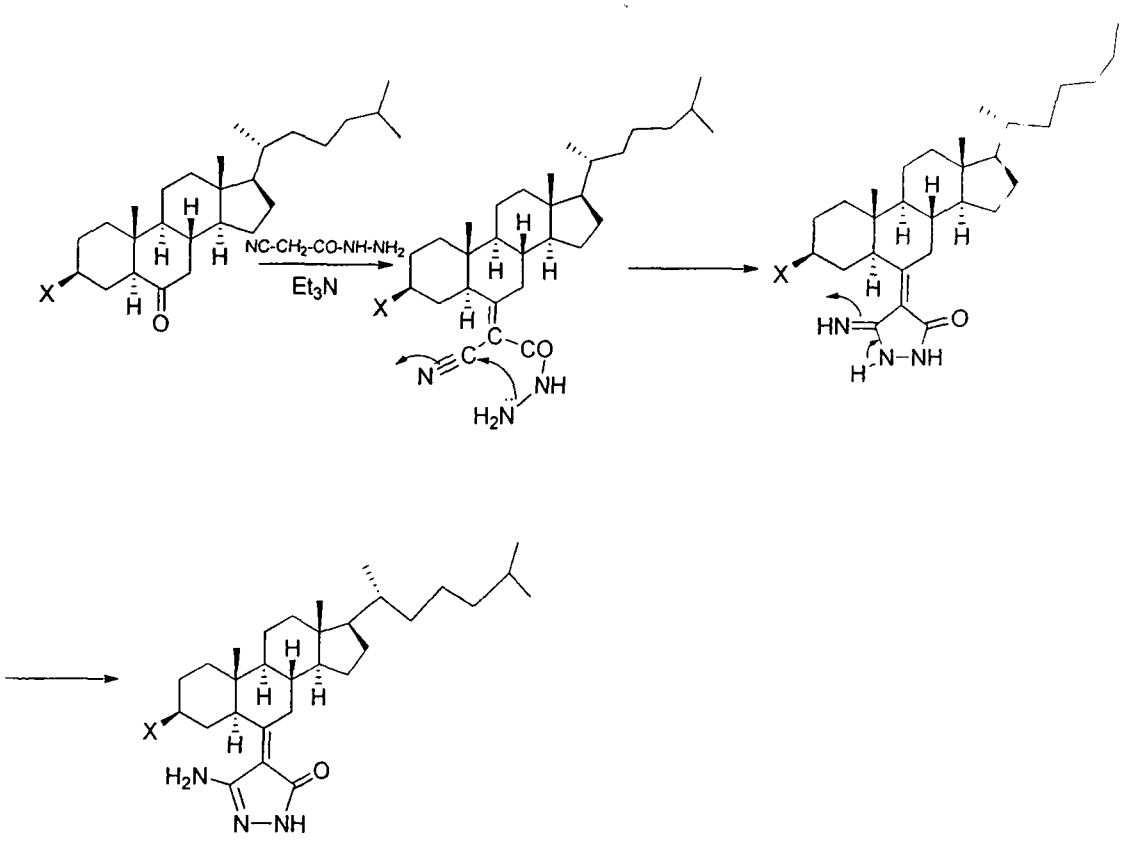
Characterization of compound 85 as 3β-chloro-6-(5'-amino-3'-oxo-dihydro-4H-pyrazol-4'-ylidine)-5α-cholestane

The elemental analysis of compound **85** corresponded to the molecular formula $C_{30}H_{48}N_3ClO$ (Beilstein positive). The IR data provided evidence for the formation of the expected structure. The compound showed intense bands in the region of 3380 and 3226 cm^{-1} due to NH and NH_2 stretching vibrations, respectively.

Other important absorption bands at 1686, 1650, 1625 and 756 cm^{-1} were attributed to C=O, C=N C=C and C-Cl stretchings, respectively. Further evidence for the formation of this steroidal derivative was well supported by its ^{1}H NMR and ^{13}C NMR spectra. The ^{1}H NMR spectrum of the compound exhibited one-proton singlet at δ 8.7 for NH (exchangeable with D_2O) while as a two-proton singlet at 2.6 for NH_2 (exchangeable with D_2O). A one-proton broad multiplet centered at δ 3.9 was assigned to C3-αH (axial, $W½$ = 17 Hz). Angular and side-chain methyl protons were observed at δ 1.19 (C10-CH_3), 0.75 (C13-CH_3), 0.97 and 0.80 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 169 for *C*O, 149 for *C*=N, 142 for C_6, 118 for C'_4 and 60 for C_3, in addition to the normal signals of cholestane series. The mass spectrum was also in good agreement with its molecular formula which exhibited a strong molecular ion peak at *m/z* 501/499.

On account of the above descriptive discussion, the compound **85** can be suitably characterized as *3β-chloro-6-(5'-amino-3'-oxo-dihydro-4H-pyrazol-4'-ylidine)-5α-cholestane.*

The reaction seems to proceed by following mechanistic pathway presented in **Scheme 1**. A mechanistic rationale includes condensation between steroidal ketones and cyanoacetohydrazide leading to the formation of steroidal cyanoacetohydrazide, which later involves the nucleophilic attack of nitrogen to the C≡N changing it to C=NH that later causes the closure of the heterocyclic ring hence leading to the formation of pyrazolones.



Scheme 1. Mechanism for the formation of steroidal pyrazolones

Experimental

General

Melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were recorded on KBr pellets with Interspec 2020 FT-IR Spectrometer spectro Lab and values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were run in $CDCl_3$ on a Bruker Avance II 500 NMR Spectrometer at 500 MHz and 125 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to the TMS (H NMR) and to the solvent signal (^{13}C NMR spectra). Mass spectra were recorded on a JEOL D-300 Mass Spectrometer. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapors to check the homogeneity as well as the progress of reaction. Sodium sulfate (anhydrous) was used as a drying agent.

***3β-Chlorocholest-5-ene*:**

Freshly distilled $SOCl_2$ (20 mL) was added gradually to cholesterol (25 g) at r.t. A vigorous reaction ensured with evolution of gaseous products. When the reaction slackened, the mixture was gently heated at temperature 50-60 °C on water bath for 1 h and then poured into water with constant stirring. The yellow solid thus obtained was filtered under suction and washed several times with ice-cold water and air dried. Recrystallization from acetone gave 3β-chlorocholest-5-ene (20 g), m.p. 95-96 °C (reported, m.p. 96-97 °C).[43]

***Cholest-5-ene*:**

3β-Chlorocholest-5-ene (10 g) was dissolved in warm amyl alcohol (230 mL) and sodium metal (20 g) was added in small portions to the solution with continuous stirring over a period of 8 h. The reaction mixture was warmed occasionally. When all the sodium metal was dissolved, methanol was added and the reaction mixture was poured into water, acidified with dilute HCl and then allowed to stand overnight. A white crystalline solid thus obtained was filtered under suction and washed thoroughly with water and air dried. The crude material was recrystallized from acetone to provide cholest-5-ene as cubes (8.3 g), m.p. 88-89 °C (reported m.p. 89-91 °C).[44]

6-Nitrocholest-5-ene:

A suspension of finely powdered cholest-5-ene (6.0 g), in glacial acetic acid (50 mL) was stirred at room temperature for 5 min. Fuming nitric acid (15 mL; d, 1.52) was rapidly added. Sodium nitrite (3 g) was added gradually over a period of 1 h with stirring and stirring was continued for 2 h. The temperature of the reaction mixture

was kept between 20-25 °C by external cooling. Cold water was added and a yellow solid thus obtained was filtered under suction, washed thoroughly with water and air dried. Recrystallization from methanol furnished 6-nitrocholest-5-ene, m.p. 119-120 °C (reported m.p. 120-121 °C).[45]

***5α-Cholestan-6-one* (80):**

6-Nitrocholest-5-ene (6 g) was dissolved in glacial acetic acid (200 mL) by heating and to this solution zinc dust (12 g) was added in small portions. After the initial exothermic reaction had subsided, the suspension was heated under reflux for 4 h, and water (12 mL) was added during the course of reaction. The solution was then filtered and the residue was washed with 40 mL warm acetic acid. To the filtrate water was added till turbidity developed and it was allowed to stand overnight at room temperature. The crystalline material thus separated was filtered under suction and washed thoroughly with water in order to remove zinc acetate. The organic solid was air dried and its recrystallization from methanol afforded 5α-cholestan-6-one (**90**), m.p. 96-98 °C (reported m.p. 98-100 °C).[40]

***3β-Acetoxycholest-5-ene*:**

A mixture of cholesterol (100 g), pyridine (150 mL, freshly distilled over KOH) and freshly distilled acetic anhydride (100 mL) was heated on a water bath for 2 h. The reaction mixture was poured into ice cold water and solid mass thus obtained was filtered under suction, washed thoroughly with water until free from pyridine and then air-dried. Recrystallization of the crude product from acetone gave 3β-acetoxycholest-5-ene (95 g), m.p. 114 °C (reported, m.p. 115-116 °C).[41]

3β-Acetoxy-6-nitrocholest-5-ene:

To a cooled mixture of 3β-acetoxycholest-5-ene (10 g) and conc. nitric acid (250 mL), sodium nitrite (10 g) was gradually added with constant stirring over a period of about 45 minutes. After complete addition of sodium nitrite stirring was continued for additional 2 h. Cold water (300 mL) was added to reaction mixture, yellow solid material separated out. The whole mass was extracted with diethyl ether. The ethereal layer was washed with water, $NaHCO_3$ solution (5%) (until washings become pink), water and dried over anhydrous sodium sulfate. Removal of the solvents provided the nitro compound as an oil which was crystallized from methanol (6.5 g), m.p. 104 °C. (reported m.p. 102-104 °C).[41]

3β-Acetoxy-5α-cholestan-6-one (**81**):

3β-Acetoxy-6-nitrocholest-5-ene (6.0 g) was dissolved in glacial acetic acid (120 mL) by warming the mixture and zinc dust (12.0 g) was added in small portions with shaking. The suspension was heated under reflux for 4 h and water (12 mL) was added at regular intervals during the course of reaction. The hot solution was filtered, cooled to room temperature and diluted with large excess of ice-cold water and extracted with diethyl ether. The ethereal solution was washed with water, $NaHCO_3$ solution (5%), again with water and dried over anhydrous sodium sulfate. Evaporation of solvents provided the acetoxy ketone as an oil which was crystallized from methanol (4.2 g) m.p. 128-129 °C (reported m.p. 127-128 °C).[41]

3β-Chloro-6-nitrocholest-5-ene:

To a well stirred mixture of 3β-chlorocholest-5-ene (14 g), glacial acetic acid (100 mL) and fulming nitric acid (28 mL; d, 1.52) at temperature below 20 °C, was added sodium nitrite (3 g) gradually over a period of 1 h. After the complete addition of sodium nitrite, the mixture was further stirred for 2 h. Now ice-cold water was added to it. The yellowish solid thus separated was filtered, washed with cold water and air dried. The crude product was recrystallized from methanol to give 3β-chloro-6-nitrocholest-5-ene, m.p. 150-52 °C (reported m.p. 153 °C).[42]

3β-Chloro-5α-cholestan-6-one (**82**):

A mixture of 3β-chloro-6-nitrocholest-5-ene (8 g) and glacial acetic acid (160 mL) was heated just to get a clear solution. The zinc dust (16 g) was added gradually in small portions with constant shaking. The suspension was heated under reflux for 4 h and water (16 mL) was added at regular intervals during the course of reaction. The hot solution was filtered and the filtrate was diluted with large excess of ice-cold water. The organic matter was extracted with diethyl ether and ethereal layer was washed successively with water, sodium bicarbonate solution (5 %) and again with water and dried over anhydrous sodium sulfate. Evaporation of the solvents furnished the ketone as an oil which was crystallized from methanol (5.8 g), m.p. 128-129 °C (reported m.p. 127-128 °C).[42]

General procedure for the synthesis of steroidal pyrazolone derivatives **(83-85):**

To a solution of steroidal ketones (**80-82**) (1 mmol) in absolute ethanol (15 mL), cyanoacetohydrazide (1 mmol) was added followed by the addition of few drops of triethyl amine. The reaction mixture was refluxed for 18-24 h. The progress of reaction was monitored by TLC. After completion of reaction the solvent was removed under reduced pressure. Now it was taken in diethyl ether, washed with water and dried over anhydrous sodium sulfate. Removal of solvents gave the crude product which was recrystallized from methanol to furnish corresponding 6-(5'-amino-3'-oxo-dihydro-4*H*-pyrazol-4'-ylidine)-5α-cholestane derivatives **(83-85).**

6-(5'-Amino-3'-oxo-dihydro-4H-pyrazol-4'-ylidine)-5α-cholestane (83)

Yield (75%); m.p. 134-135 °C; Anal. Calcd for $C_{30}H_{49}N_3O$: C, 77.04, H, 10.56, N, 8.98; found; C, 77.05, H, 10.52, N, 8.95; IR (KBr) ν cm^{-1} 3385, 3230 (NH, NH_2), 1691 (CONH), 1657 (C=N), 1622 (C=C), 1378 (C-N); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.9 (s, 1H, CON*H*, exchangeable with D_2O), 2.3 (s, 2H, N*H*$_2$, exchangeable with D_2O), 2.0 (dd, 1H, J =7.55, 4.52 Hz, C_5-H), 1.19 (s, 3H, C_{10}-C*H*$_3$), 0.75 (s, 3H, C_{13}-C*H*$_3$), 0.96 and 0.83 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173, 150, 140, 119, 56, 56, 54, 42, 40, 39, 38, 37, 36, 36, 36, 34, 32, 30, 28, 28, 27, 27, 26, 25, 24, 23, 23, 19, 14, 13; MS (EI): (*m/z*) 467 [$M^{+\cdot}$].

3β-Acetoxy-6-(5'-amino-3'-oxo-dihydro-4H-pyrazol-4'-ylidine)-5α-cholestane (84)

Yield (80%) m.p. 159-160 °C; Anal. Calcd for $C_{32}H_{51}N_3O_3$: C, 73.10, H, 9.78, N, 7.99; found; C, 73.12, H, 9.81, N, 8.0; IR (KBr) ν cm^{-1} 3390, 3210 (NH, NH_2), 1736 ($OCOCH_3$), 1689 (CONH), 1655 (C=N), 1628 (C=C), 1376 (C-N), 1240 (C-O); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.6 (s, 1H, CON*H*, exchangeable with D_2O), 4.7 (m, 1H, $C_3\alpha$-H, W ½=15 Hz, axial), 2.5 (s, 2H, N*H*$_2$ exchangeable with D_2O,), 2.3 (dd, 1H, J =7.55, 4.52 Hz,C_5-*H*), 2.03 (s, 3H, OCOC*H*$_3$), 1.18 (s, 3H, C_{10}-C*H*$_3$), 0.70 (s, 3H, C_{13}-C*H*$_3$), 0.97 and 0.83 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171, 167, 154, 145, 118, 75, 56, 56, 54, 42, 40, 39, 38, 37, 36, 36, 36, 34, 32, 30, 28, 28, 27, 26, 25, 24, 23, 23, 22, 19, 14, 13; MS (EI): (*m/z*) 525 [$M^{+\cdot}$].

3β-Chloro-6-(5'-amino-3'-oxo-dihydro-4H-pyrazol-4'-ylidine)-5α-cholestane (85)

Yield (78%) m.p. 147-148 °C; (Beilstein positive); Anal. Calcd for $C_{30}H_{48}N_3ClO$: C, 71.75, H, 9.63, N, 8.37; found; C, 71.79, H, 9.59, N, 8.34; IR (KBr) ν cm^{-1} 3380, 3226 (NH, NH_2), 1685 (CONH), 1650 (C=N), 1625 (C=C), 1371 (C-N), 756 (C-Cl); ^1H NMR ($CDCl_3$, 500 MHz) 8.7 (s, 1H, CON*H*, exchangeable with D_2O), 3.9 (m, 1H, $C_3\alpha$-H, W ½ = 17 Hz, axial), 2.6 (s, 2H, N*H*$_2$, exchangeable with D_2O), 2.3 (dd, 1H, J

=7.55, 4.52 Hz ,C_5-H), 1.19 (s, 3H, C_{10}-CH_3), 0.75 (s, 3H, C_{13}-CH_3), 0.97 and 0.80 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169, 149, 142, 118, 60.2, 56, 56, 54, 42, 40, 39, 38, 37, 36, 36, 36, 34, 32, 30, 28, 28, 27, 26, 25, 24.8, 23.8, 23.2, 19.9, 14.08, 13; MS (EI): (*m/z*) 501/499 [$M^{+\cdot}$].

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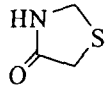
CHAPTER-4

Steroidal Thiazolidinones

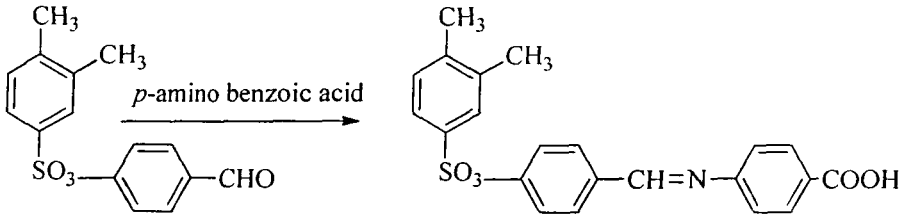
Theoretical

Thiazolidinones and their derivatives display a large variety of activities such as antibiotic, diuretic, organoleptic, tuberculostatic, antileukemic and antiparasitical.[1,2] Thiazolidinones (**1**) are classified as five membered heterocyclic compounds containing one nitrogen, one sulfur and three carbon atoms including a carbonyl group. Our continuous interest in the synthesis of steroidal compounds prompted us to prepare new steroidal thiazolidinone derivatives from steroidal ketones and simultaneously study the biological behavior like antimicrobial, anticancer and antioxidant activity.

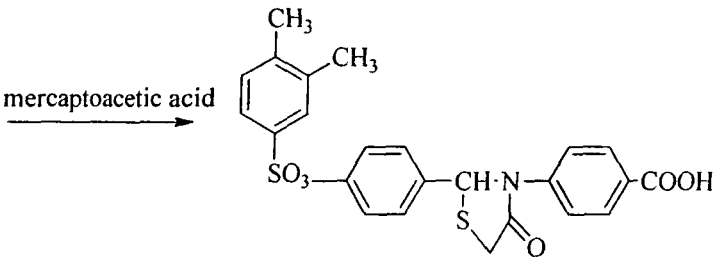


(1)

Moawad *et al.*[3] reported that reaction of Schiff base (**3**) with mercaptoacetic acid in dry benzene afforded thiazolidinone (**4**). Schiff base (**3**) was obtained by the condensation of 3,4-xylene sulfonate ester of *p*-hydroxy benzaldehyde (**2**) with *p*-amino benzoic acid.

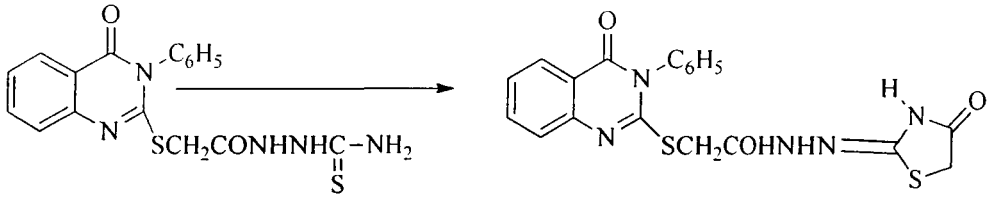


(2) **(3)**



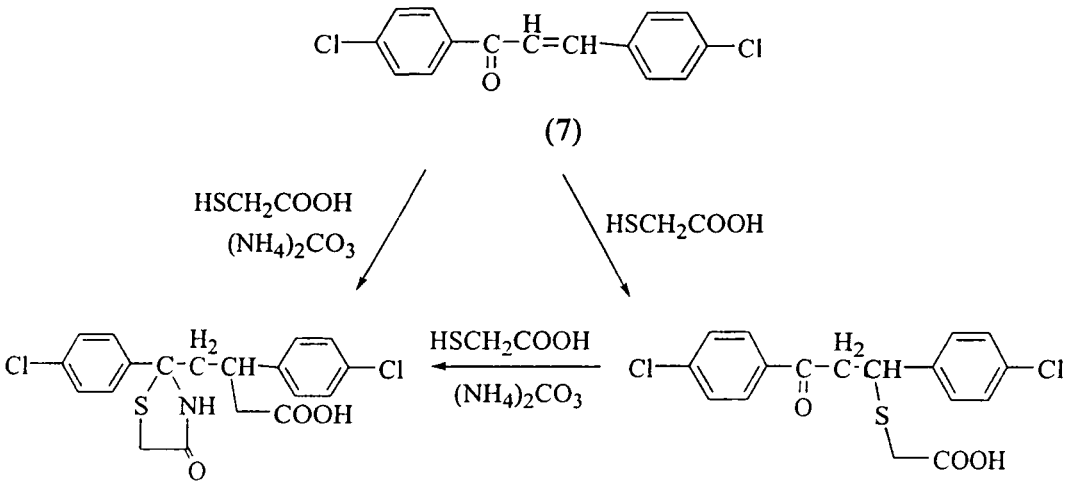
(4)

Gursoy *et al.*[4] prepared new thiazolidinone (**6**) by the reaction of quinazoline derivative (**5**) with mercaptoacetic acid.



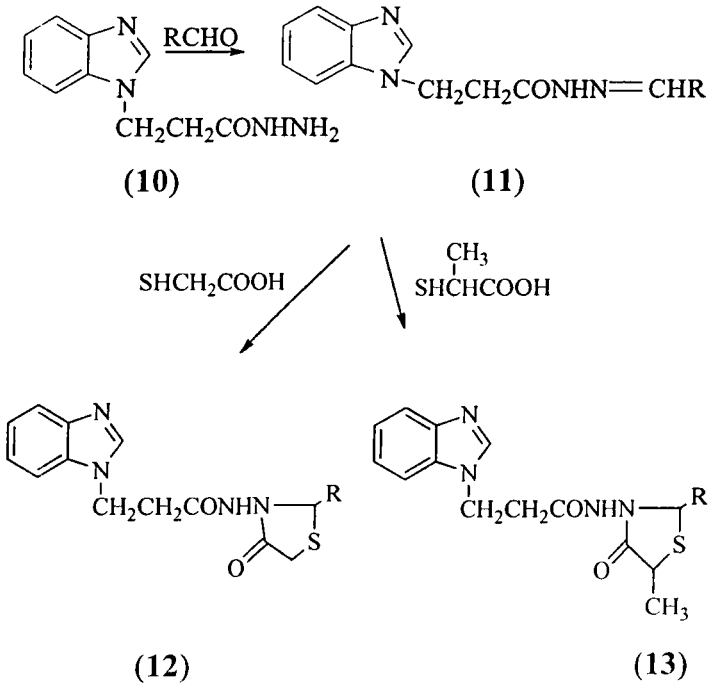
(5) (6)

Ansari *et al.*[5] synthesized 4-thiazolidinone (9) starting form chalcone (7), by two different routes as shown in the following scheme.

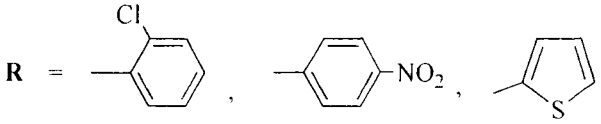


(7) (9) (8)

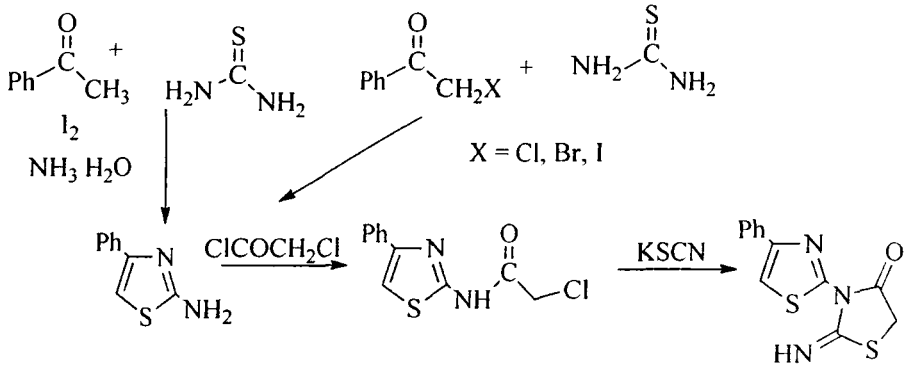
El-masry *et al.*[6] reported that condensation of compound **10** with aromatic and heterocyclic aldehydes in absolute ethanol afforded the corresponding Schiff bases **11**). The cyclocondensation of substituted Schiff bases with mercaptoacetic acid/ thiolactic acid afforded the corresponding thiazolidinones **12** and **13**.



(10) **(11)** **(12)** **(13)**

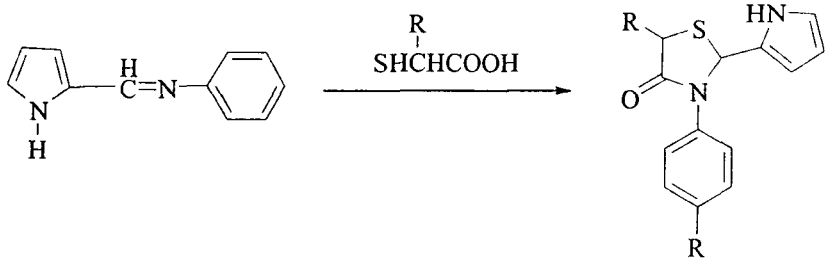


Anthonsen *et al.*[7] synthesized 2-imino-3-(4-phenylthiazol-2-yl)-thiazolidin-4-ones (**16**) *via* the key intermediate, 2-amino-4-phenylthiazoles (**14**) which was subjected to with chloroacetyl chloride to produce 2-chloro-acetamido-4-phenylthiazole (**15**) following its treatment with potassium thiocyanate in refluxing acetone to afford the required product **16**.



(**14**) (**15**) (**16**)

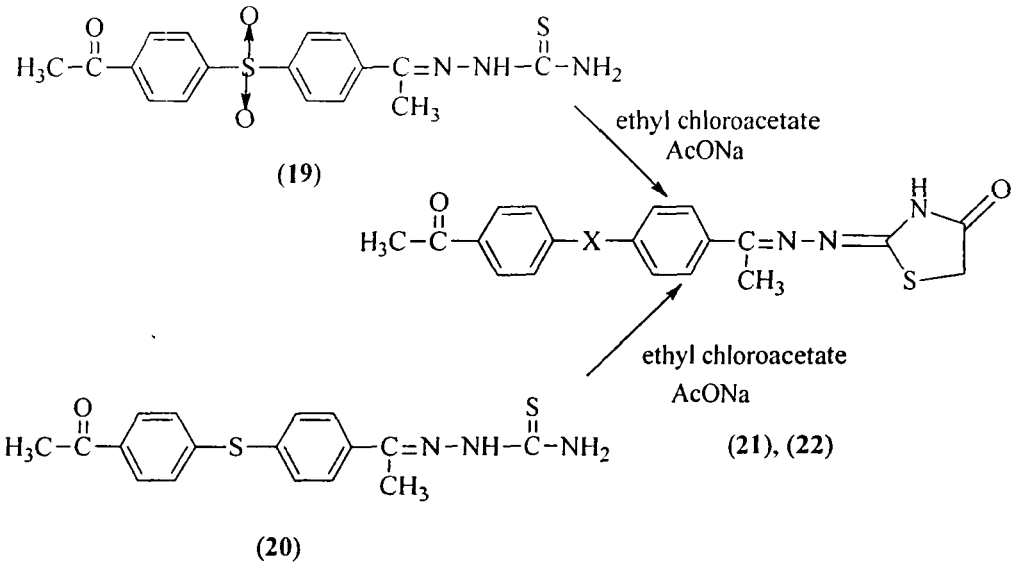
Ocal *et al.*[8] synthesized substituted 4-thiazolidinones (**18**) by the reaction of aldimine (**17**) with mercaptoacetic acids which were obtained by the condensation of pyrrole-2-carbaldehyde with aromatic amine.



(**17**) (**18**)

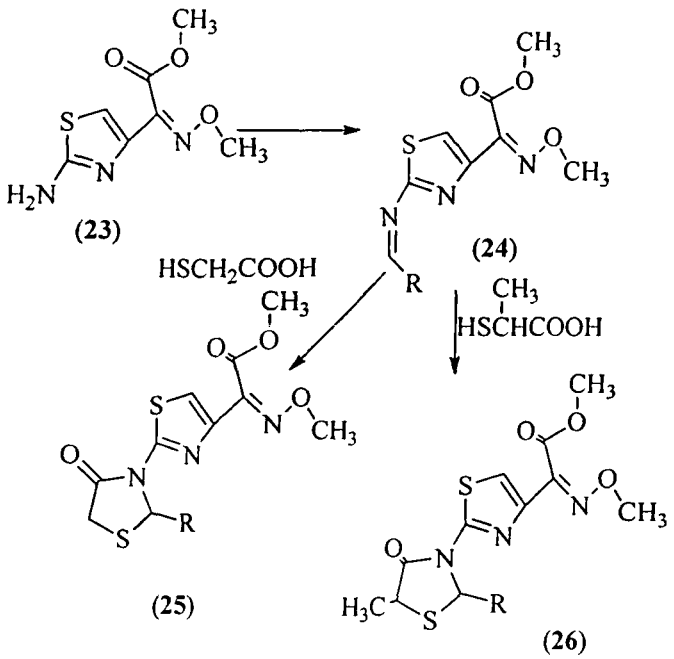
R = H, CH_3

Abbady *et al.*[9] obtained 4-acetylthiosemicarbazone-4-acetyldiphenyl sulfone (**19**) and 4-acetylthiosemicarbazone-4-acetyldiphenyl sulfide (**20**) which on further reaction with ethylchloroacetate in the presence of fused AcONa gave 4-(4"-thizolidinone-2"-acetylazino)-4'-acetyldiphenyl sulpfone (**21**) [X = SO_2] and 4-(4"-thizolidinone-2"-acetylazino)-4'-acetyldiphenyl sulfide (**22**) [X = S], respectively.



(19) (20) (21), (22)

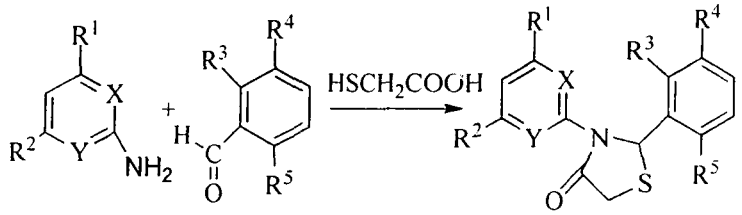
Parekh *et al.*[10] prepared substituted Schiff bases (**24**) by the treatment of 2-amino-4-(α-methoxyiminocarbomethoxymethyl)-thiazole (**23**) with aromatic aldehyde. The compound **24** on further reaction with mercaptoacetic acid and methyl substituted mercaptoacetic acid in dry benzene furnished desired thiazolidinones (**25**) and (**26**), respectively. The products were evaluated for their *in vitro* growth inhibiting activity against several microbes. Some of them showed significant anti-tubercular and antifungal activity.



(23) (24) (25) (26)

R = Ar

Monforte *et al.*[11] reported the synthesis of 2,3-diaryl-1,3-thiazolidin-4-ones (**29**) by reacting an aromatic aldehyde (**28**) with an equimolar amount of (hetero) aromatic amine (**27**) in the presence of an excess of mercaptoacetic acid. The microwave-irradiation dramatically shortened the reaction time, affording the desired products in high yields.



(27) **(28)** **(29)**

R^1 = Me, OMe, Br **X** = CH, N

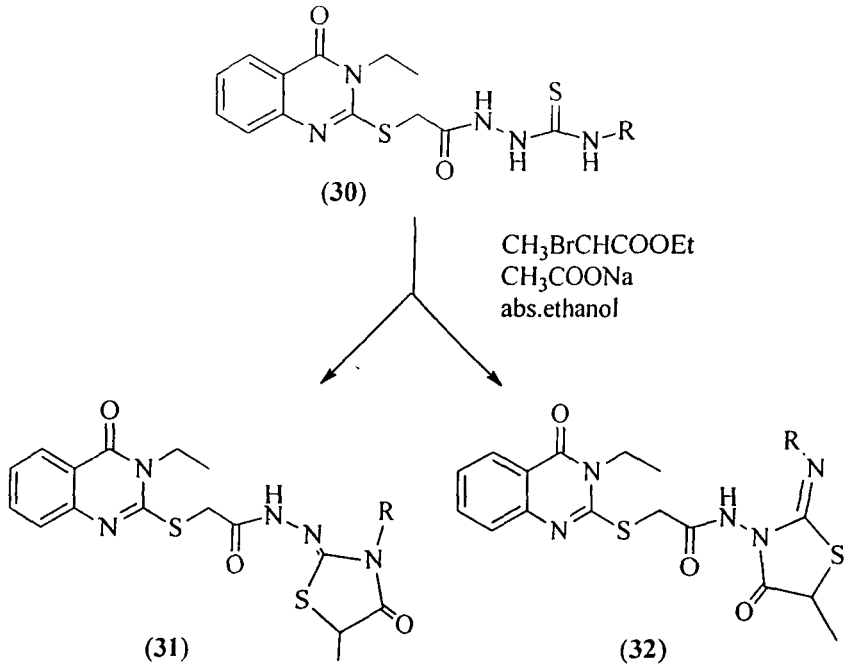
R^2 = H, Me **Y** = CH, N

R^3 = Cl, F

R^4 = H, Me

R^5 = Cl, F

Terzioglu and Glu[12] synthesized the target compounds, 2-(3-ethyl-4(3*H*)-quainazolinone-2-ylmercaptoacetylhydrazono)-3-alkyl/aryl-5-methyl-4thiazolidinones (**31**) and 2-arylimino-3-(3-ethyl-4(3*H*)-quinazolinone-2-ylmercaptoa-cetylamino)-5-methyl-4-thiazolidinones (**32**) by the cyclization of 3-ethyl-4(3*H*)-quinazolinone-2-ylmercaptoacetyl)-4-alkyl/arythiosemicrabazides (**30**) with ethyl-2-bromopropionate in presence of anhydrous sodium acetate in absolute ethanol.

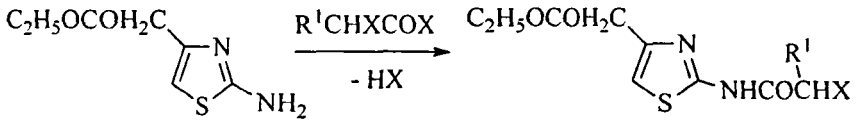


(30)

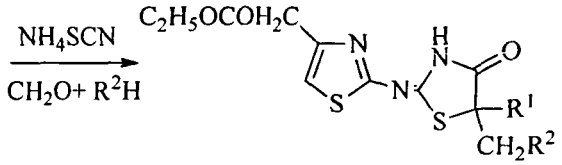
(31) (32)

R= CH_3, C_6H_{11}

Altintas *et al.*[13] synthesized 5-(N,N-disubstitutedaminomethyl-2-[(4-carbethoxymethylthiaxol-2-yl)imino] 4-thiazolidinones compound **35** by refluxing 4-carbethoxymethyl-2-[(*α*-chloropropoinyl/*α*-bromobutyryl/*α*-chloro-(-phenyl)acetyl)amino] thiazoles (**34**) with ammonium thiocyanate. The product (**35**) also evaluated for antibacterial and antifungal activities.

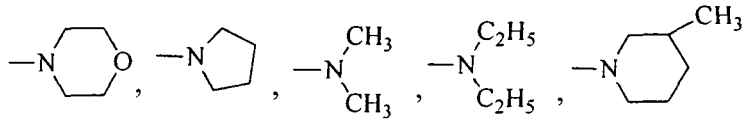


(33) (34)



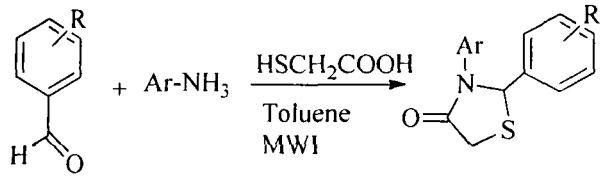
(35)

R^1 = CH_3, C_2H_5, C_6H_5

R^2 = 

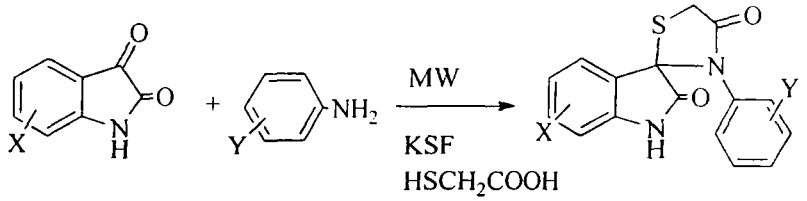
X = Cl, Br

Sriram *et al.*[14] reported the synthesis of 1,3-thiazolidin-4-ones (**38**) by reacting substituted benzaldehyde (**36**) with equimolar amount of an appropriate substituted aromatic amine (**37**) in the presence of an excess of mercaptoacetic acid in toluene under microwave irradiation. Unlike the conventional methods (48 h, 30-70 %), microwave-assisted reactions were very facile (6-8 min) and provided very good yields (64-82 %). [**Ar** = 4-F-C_6H_4, **R** = 4-Cl, 2-Cl]



(36) **(37)** **(38)**

Arya *et al.*[15] prepared *spiro* [indole-thiazolidines] (**41**) by the multicomponent condensation between indole-2,3-dione (**39**), aromatic amine (**40**) and marcaptoacetic acid using montmorllonite KSF as solid support in 85-90 % yield in 4-5 min under MW conditions.

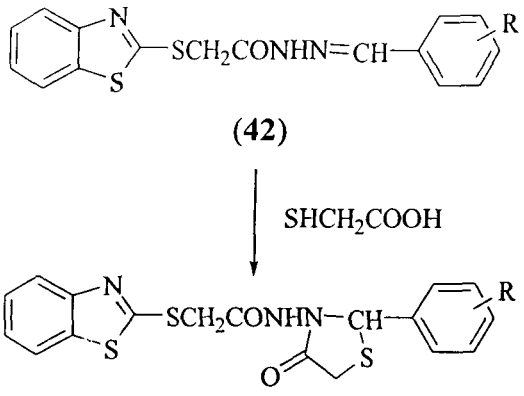


(39) **(40)** **(41)**

X = H, 5-Cl, 5-CH_3

Y = OCH_3

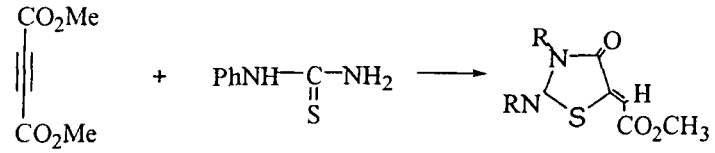
Desai *et al.*[16] synthesized 4-thiazolidinones (**43**) in a good yield from the heterocyclization reaction of 2-(benzothiazol-2-ylthio)-N'-benzylideneacetohydrazide (**42**) with mercaptoacetic acid in DMF in the presence of catalytic amount of anhydrous $ZnCl_2$ under microwave irradiation.



(42)

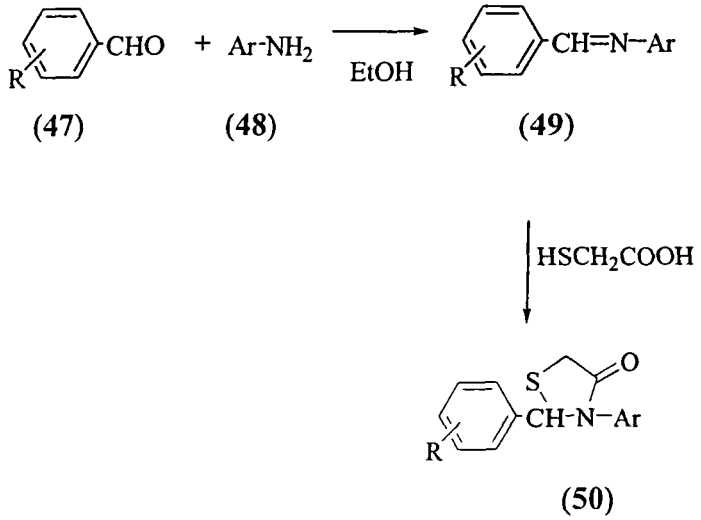
R = 4-NO_2, CH_3 (43)

Nagarajan[17] synthesized *E*-thiazolidinone (**46**) by the cyclization of acetylene dicarboxylic ester (**44**) with thiocarbamoyl derivative (**45**).



(44) (45) (46)

Sahu *et al.*[18] reported that condensation of substituted benzaldehydes (**47**) with primary aryl amines (**48**) gave a series of Schiff bases (**49**) which, on reaction with mercaptoacetic acid, resulted in the formation of the corresponding 4-thiazoldinones (**50**).



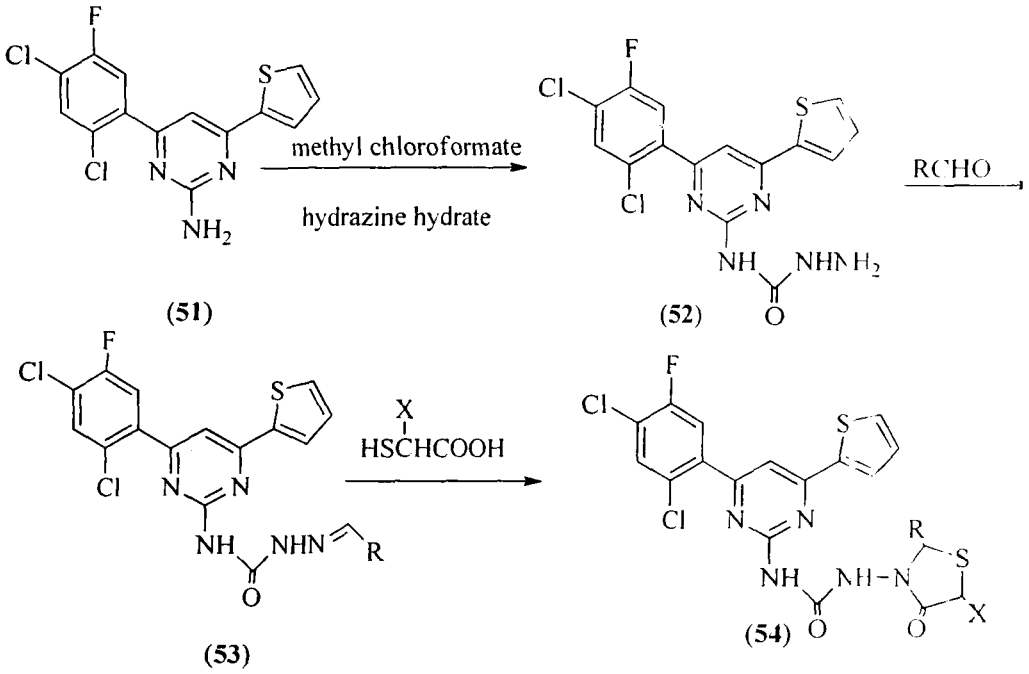
(47) (48) (49)

(50)

Ar = 4-NO_2, Phenyl, Naphthyl

R = 2-OH, 4-$N(CH_3)_2$, 4-NO_2, 4-Cl and 4-OCH_3

Shah and Desai[19] synthesized 2-(substituted phenyl)-3-[4-(2,4-dichloro-5-fluorophenyl)-6-(2-thienyl)pyrimidine-2-yl-ureido] 5*H*/ methyl/carboxy methyl-4-thiazolidinones (**54**) starting with compound **51** and employing the following reaction scheme.

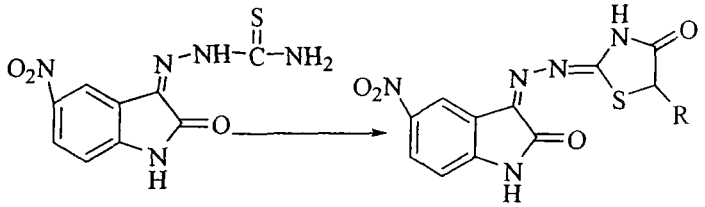


(51) (52) (53) (54)

R = aryl

X = H, CH_3, CH_2COOH

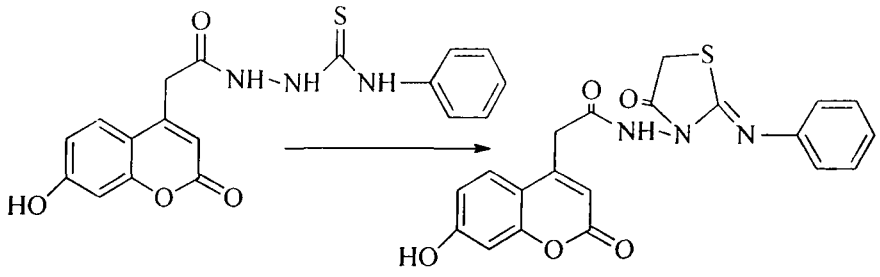
Terioglu *et al.*[20] synthesized series of 5-nitro-3-[(5-nonsubstituted/methyl-4-thiazolidinone-2-ylidene) hydrazono]-1*H*-2-indolinones (**56**) by reaction of 5-nitro-1*H*-indole-2,3-dione-3-thiosemicarbazone (**55**) with ethyl bromoacetate or ethyl 2-bromopropionate.



(55) (56)

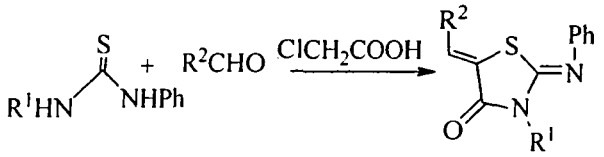
R = H, CH_3

Cacic *et al.*[21] reported the cyclizaiton of thiosemicarbazide (**57**) with chloroacetylchloride in chloroform which afforded thiazolidinone derivative (**58** .



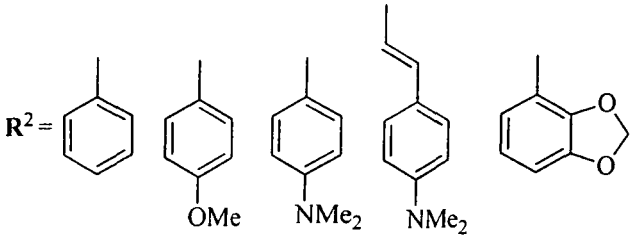
(57) (58)

Mir *et al.*[22] reported a rapid and easy solvent free one-pot synthesis of 5-arylidene-2-imino-4-thiazolidinones (**61**) by condensation of the thiourea derivatives (**59**) with chloroacetic acid and aldehydes (**60**) under microwave-irradiation.

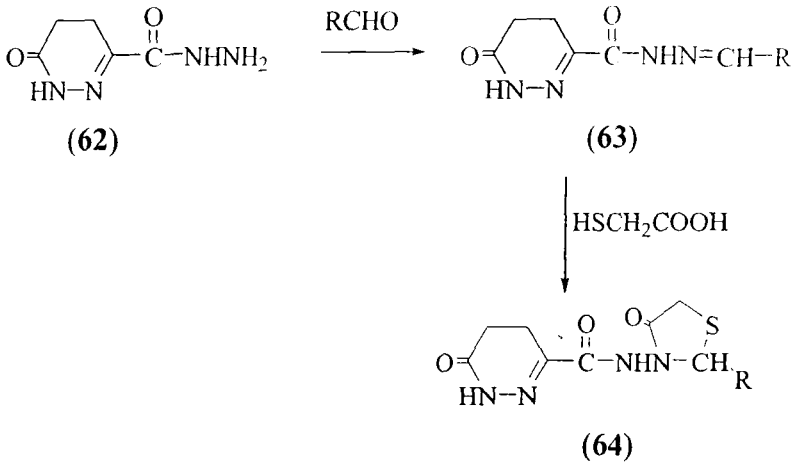


(59) (60) (61)

$\mathbf{R^1}$= Ph, 4-Methylpyridin-2-yl



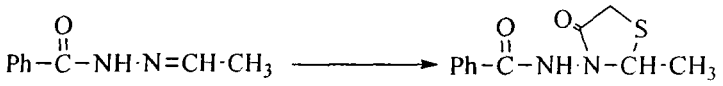
Cao *et al.*[23] reported the reaction of 1,4,5,6-tetrahydro-6-pyridazinone-3-carboxylic acid hydrazides (**62**) with aromatic aldehydes to afforded hydrazones (**63**). Hydrazones (**63**) which on reaction with mercaptoacetic acid in DMF in the presence of anhydrous $ZnCl_2$ afforded 1,3-thiazolidinone derivative (**64**).



(62) (63) (64)

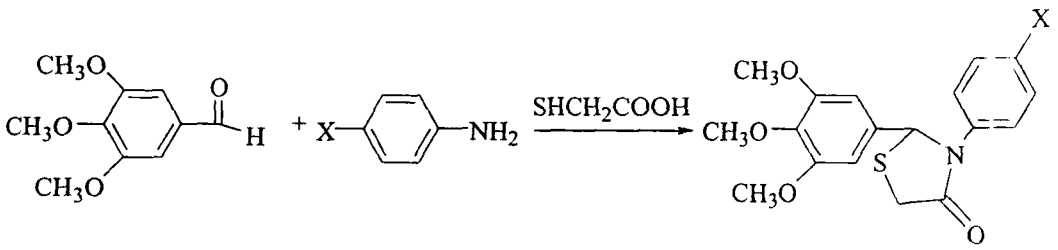
R = C_6H_5, 4-$MeOC_6H_5$, 4-$NMe_2C_6H_5$, 2-$NO_2C_6H_4$, 3-$NO_2C_6H_4$, 3-OMe-4-OH-C_6H_3

Rollas and Kucukguzel[24] synthesized 4-thiazolidinones (**66**) by reacting hydrazones (**65**) with mercaptoacetic acid/thiolactic acid.



(65) (66)

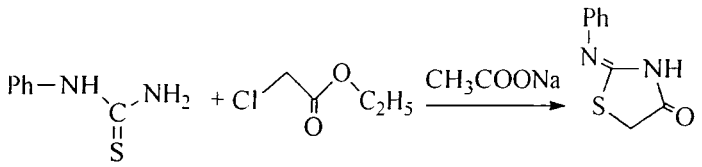
Turgut *et al.*[25] prepared 4-thiazolidinones (**69**) by the Katti carbodiimide (DCC) mediated one-pot three component condensation reaction of substituted aromatic amines (**68**), substituted aldehyde (**67**) and mercaptoacetic acid.



(67) (68) (69)

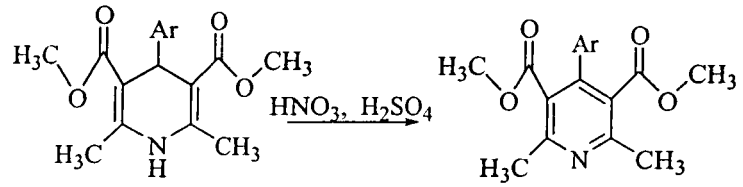
X = CH_3, Cl, OC_6H_5, OCH_3, OC_2H_5

Shiradkar[26] reported that various phenyl-thiourea derivatives (**70**) on treatment with ethyl chloraoacetate (**71**) and fused sodium acetate in absolute ethanol gave 2-phenylimino-4-thiazolidinones (**72**).

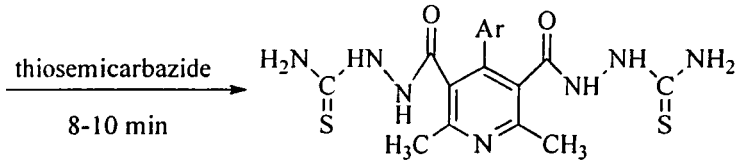


(70) (71) (72)

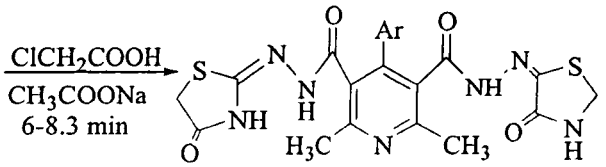
Ameta *et al.*[27] reported a fast and facile procedure for the synthesis of pyridothiazolidinone (**76**) starting from dihydropyridine (**73**). Oxidation of (**73**) with nitrating mixture (HNO_3/H_2SO_4) produced the anticipated 2,6-dimethylpyridine derivatives (**74**), which were subsequently condensed thiosemicarbazide in ethanol to produce the key intermediate 2,2-[4-(4-substitutedphynely)-2,6dimethylpyridine-3,5di-yl]-dicarbonyl dihydrazine carbothioamides (**75**). Compound (**75**) finally provided (**76**) on reacting with $ClCH_2COOH$ and CH_3COONa. Their reactions were carried by conventional as well as microwave method. The potent antimicrobial effects of the synthesized compounds were also investigated.



(73) (74)



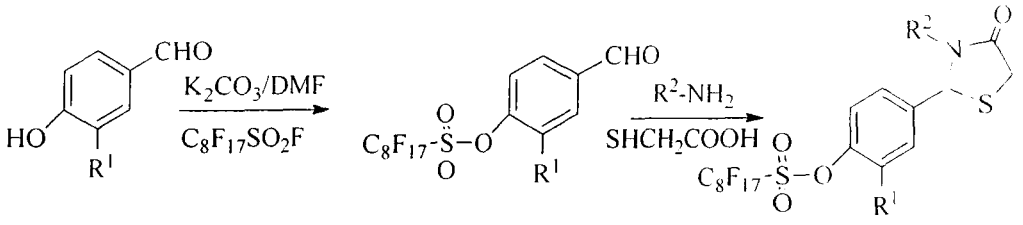
(75)



(76)

Ar = 4-F-C_6H_4, 4-Cl-C_6H_4, 4-$OCH_3C_6H_4$, 4-NO_2-C_6H_4, C_6H_5

Yan *et al.*[28] reported the construction of 4-thiazolidnone ring (**79**) by a three component reaction of fluorous benzaldehydes (**78**), an amine and mercaptoacetic acid. Compound **78** was readily prepared from **77**.

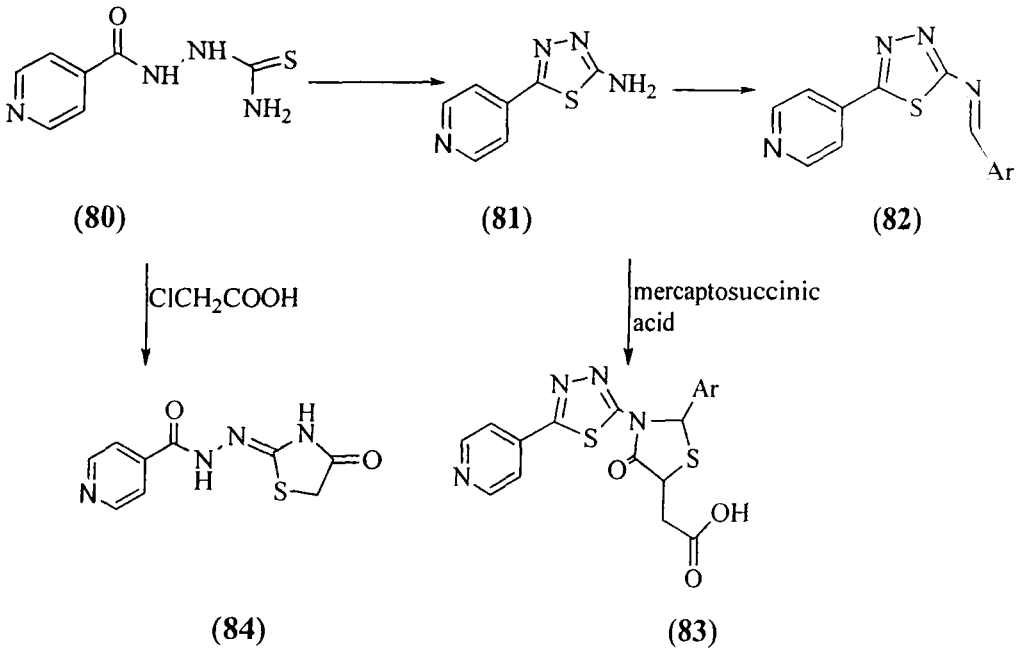


(77) (78) (79)

$\mathbf{R^1}$ = H, OMe

$\mathbf{R^2}$ = n-Butyl, 4-Me-Ph

Talesara *et al.*[29] synthesized 2-amino-5-(4'-pyridyl)-1,3,4-thiadiazole (**81**) by cyclization of isonicotinoylthiosemicarbazide (**80**) with conc. sulfuric acid. When **81** was refluxed with various aldehydes [Ar-CHO] it gave the corresponding arylidene derivatives (**82**) which on further treatment with mercaptosuccinic acid furnished thiazolidinone derivatives (**83**). Another thiazolidinone derivative, isonicotinoylhydrazido-1,3-thiazolidinone (**84**), was obtained by the treatment of **80** with chloroacetic acid in the presence of sodium acetate. [**Ar** = 4-$OCH_3C_6H_4$. 3,4,5-$OCH_3C_6H_2$, 3-$NO_2C_6H_4$, 4-$NO_2C_6H_4$, 4-$(CH_3)_2NHC_6H_4$, C_6H_5, C_4H_3O(2-furyl)].

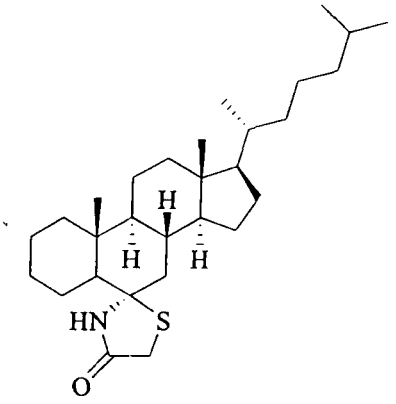


(80) (81) (82)

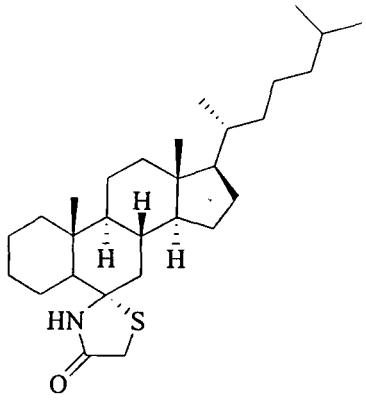
(84) (83)

Literature has revealed that very few steroidal thiazolidinones have been prepared and studied so far. Shafiullah and Ali[30] reported the synthesis and mass spectral studies of the *spiro* thiazolidinones (**85**) and (**86**). Steroidal thiazolidinones[31]

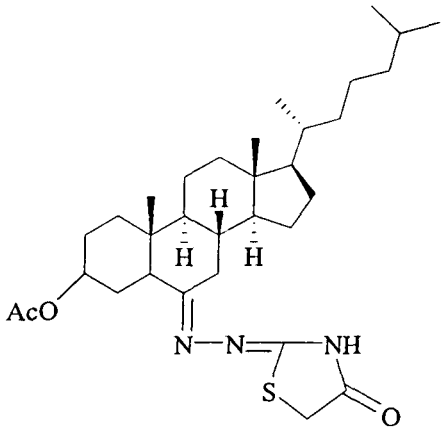
of cholestane series (**87**) and (**88**) were also prepared by cyclising the respective thiosemicarbazones with $ClCH_2COOH$.



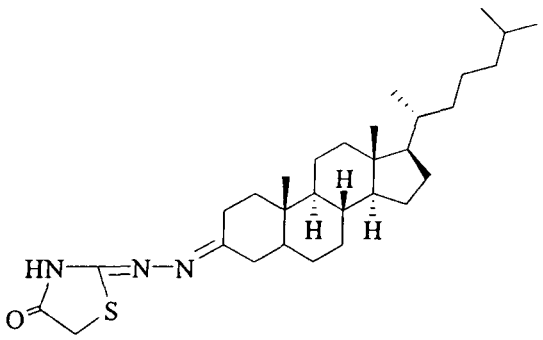
(85)



(86)

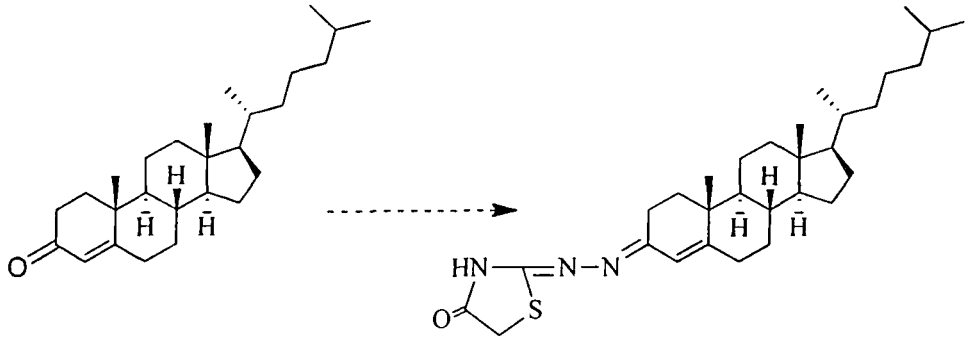


(87)



(88)

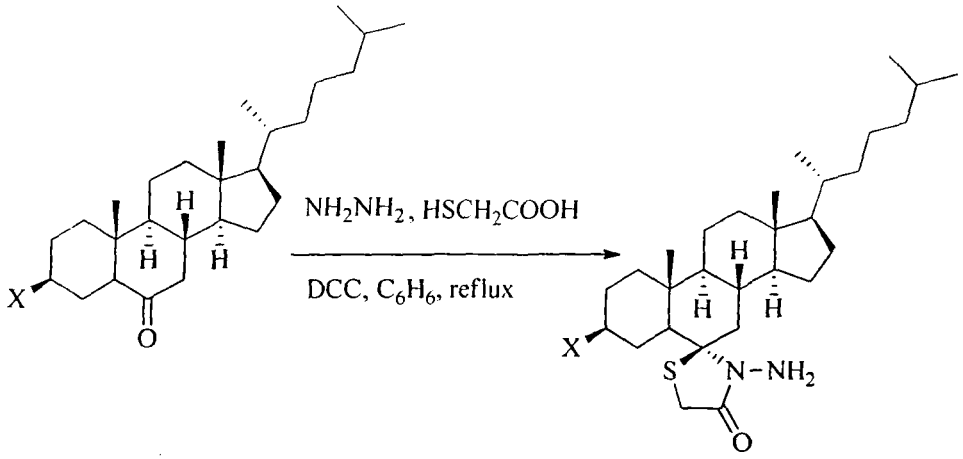
Our lab[32] prepared 3-diazo(4′-thiazolidinone)cholest-4-en (**90**) from cholest-4-en-3-one (**89**) in two steps reaction.



(89) **(90)**

Discussion

Thiazolidinones have been reported to show versatile pharmacological activities. They have been reported as COX-1 inhibitor,[33] anti-inflammatory,[34] antiproliferative,[35,36] antihistaminic,[37] anti-HIV,[38,39] hypnotic,[40] anaesthetic,[41] antifungal,[42] anthelmintic[43] and antiviral[44] agents as well as CNS[45] stimulants. Thiazolidinones and their derivatives[46] exhibit unusually high activity against *Mycobacterium tuberculosis*. Recently, a number of thiazolidinones derivatives found to exhibit highly potent and selective anti-Platelet activating factor activity both *in vitro* and *in vivo*.[47] 2-Arylimino-4-thiazolidinone derivatives have also showed antibacterial,[48,49] antifungal,[50] anticonvulsant[51,52]and anticancer[53] activities. Furthermore, most of the 4-thiazolidinones and their benzylidene derivatives display a large variety of activities such as antibiotic, diuretic, organoleptic, tuberculoststic, antileukemic and antiparasital.[54,55] The pharmacological properties of 4-thiazolidinones encouraged our interest in synthesizing several new compounds featuring various heterocyclic rings, attached to 4-thiazolidinone moiety. For a long time imines have been used successfully in the synthesis of nitrogen containing heterocycles.[56] As a part of our aim to search for biologically active heterocycles containing sulfur and nitrogen, we have undertaken the synthesis of some steroidal thiazolidinones eg. 5α-cholestan-(6*R*)-*spiro*-6,3′-amino-1′,3′,4′-thiazolidinone (**94**), 3β-acetoxy-5α-cholestan-(6*R*)-*spiro*-6,3′-amino-1′,3′,4′-thiazolidinone (**95**) and 3β-chloro-5α-cholestan-(6*R*)-*spiro*-6,3′-amino-1′,3′,4′-thiazolidinone (**96**) starting from 5α-cholestan-6-one (**91**), 3β-acetoxy-5α-cholestan-6-one (**92**) and 3β-chloro-5α-cholestan-6-one (**93**). The structure of newly synthesized compounds has been assigned on the basis of elemental analysis and spectral (IR, ^{1}H NMR, ^{13}C NMR, MS) studies.

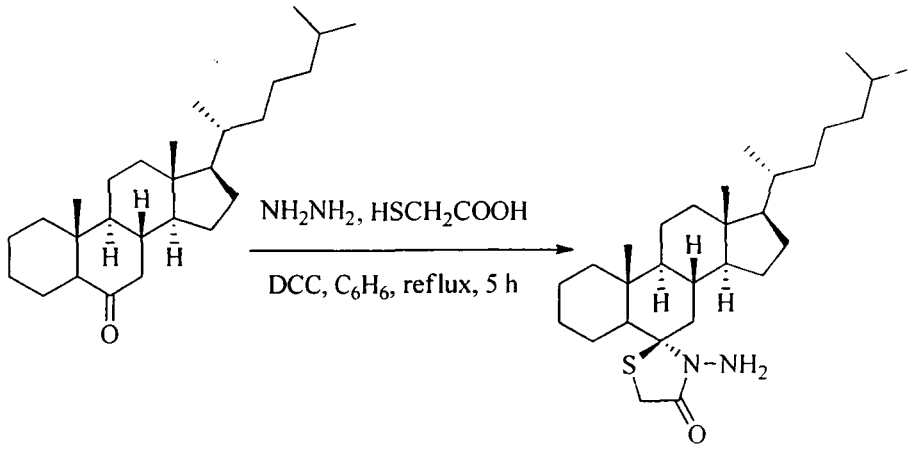


X	
H	**(91)**
OAc	**(92)**
Cl	**(93)**

X	
H	**(94)**
OAc	**(95)**
Cl	**(96)**

Reaction of 5α-cholestan-6-one (91) with hydrazine hydrate and mercaptoacetic acid: 5α-Cholestan-(6R)-spiro-6,3'-amino-1',3',4'-thiazolidinone (94):

A mixture of steroidal ketone **91**, hydrazine hydrate and mercaptoacetic acid in benzene was refluxed for 5 h in the presence of DCC in. After usual work up and recrystallization provided a single product **94**, m.p. 128-130 °C, was obtained.



(91) **(94)**

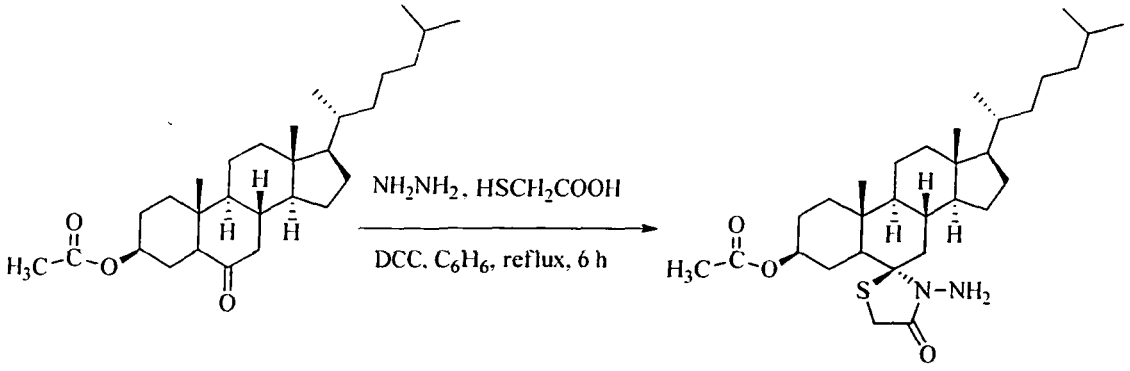
Characterization of compound 94 as 5α-cholestan-(6R)-spiro-6,3'-amino-1',3',4'-thiazolidinone:

The elemental analysis of the compound **94** corresponded to the molecular formula $C_{29}H_{50}N_2OS$. The IR data provided evidence for the formation of the expected product. The compound showed intense band in the region of 3215 cm^{-1} due to NH_2 stretching vibrations. In addition, other important absorption bands at 1645, 1229 and 674 cm^{-1} were attributed to C=O, C-N and C-S stretchings, respectively. Further evidence for the formation of compound **94** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited two singlets integrating for two protons each at δ 8.11 (exchangeable with D_2O) for NH_2 and 3.36 for methylene protones of thiazolidinone ring. Angular and side-chain methyl protons were observed at δ 1.19 (C10-CH_3), 0.75 (C13-CH_3), 0.91 and 0.85 for other methyl protons. ^{13}C NMR spectrum displayed characteristic signals at δ 173 for *C*=O, 38 for methylene carbon of thiazolidinone ring and 67 for C_6, in addition to the usual signals of cholestane series. The mass spectrum was also in agreement with its molecular formula which exhibited a strong molecular ion peak at *m/z* 474.

On account of the above descriptive discussion, the compound **94** can be suitably characterized as *5α-cholestan-(6R)-spiro-6,3'-amino-1',3',4'-thiazolidinone*.

Reaction of 3β-acetoxy-5α-cholestan-6-one (92) with hydrazine hydrate and mercaptoacetic acid: 3β-Acetoxy-5α-cholestan-(6R)-spiro-6,3'-amino-1',3',4'-thiazolidinone (95):

A mixture of steroidal ketone **92**, hydrazine hydrate and mercaptoacetic acid in the presence of DCC was refluxed in benzene for 6 h. Usual work up provided a single product **95**, m.p. 132-134 °C.



(92) (95)

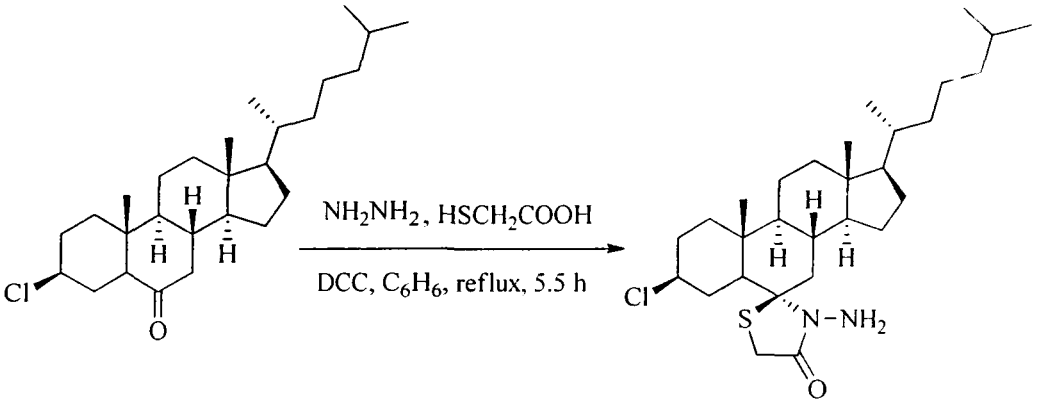
Characterization of compound 95 as 3β-acetoxy-5α-cholestan-(6R)-spiro-6,3'-amino-1',3',4'-thiazolidinone:

Compound **95** was correctly analyzed for $C_{31}H_{52}N_2O_3S$. The IR data suggested evidence the formation of the expected product. The compound showed intense band in the region of 3218 cm^{-1} due to NH_2 stretching vibrations. In addition, other important absorption bands at 1763, 1648, 1234 and 680 cm^{-1} were attributed to C=O (ester), C=O, C-N and C-S stretchings, respectively. Further evidence for the formation of compound **95** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited two singlets integrating for two protons each at δ 8.2 (exchangeable with D_2O) for NH_2 and at 3.38 for methylene protons of thiazolidinone ring. A one-proton broad multiplet centered at δ 4.7 was assigned to C3-αH (axial, $W\frac{1}{2}$ = 15 Hz) and a sharp singlet for three acetoxy group protons appeared at 2.03. Angular and side-chain methyl protons were observed at 1.19 (C10-CH_3), 0.75 (C13-CH_3), 0.91 and 0.85 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure by displaying characteristic signals at δ 176 for *C*=O, 172 for C_3, 40 for methylene carbon of thiazolidinone ring and 69 for C_6, in addition to the usual signals of cholestane nucleus. The mass spectrum was also found in good agreement with its molecular formula which exhibited a strong molecular ion peak at *m/z* 532.

On account of the above evidences, the compound **95** can be suitably characterized as *3β-acetoxy-5α-cholestan-(6R)-spiro-6,3'-amino-1',3',4'-thiazolidinone.*

Reaction of 3β-chloro-5α-cholestan-6-one (93) with hydrazine hydrate and mercaptoacetic acid: 3β-Chloro-5α-cholestan-(6R)-spiro-6,3'-amino-1',3',4'-thiazolidinone (96):

A mixture of steroidal ketone **93**, hydrazine hydrate and mercaptoacetic acid in the presence of DCC was refluxed in benzene for 5.5 h. After usual work up, a single product **96**, m.p. 136-138 °*C* was obtained.



(93) **(96)**

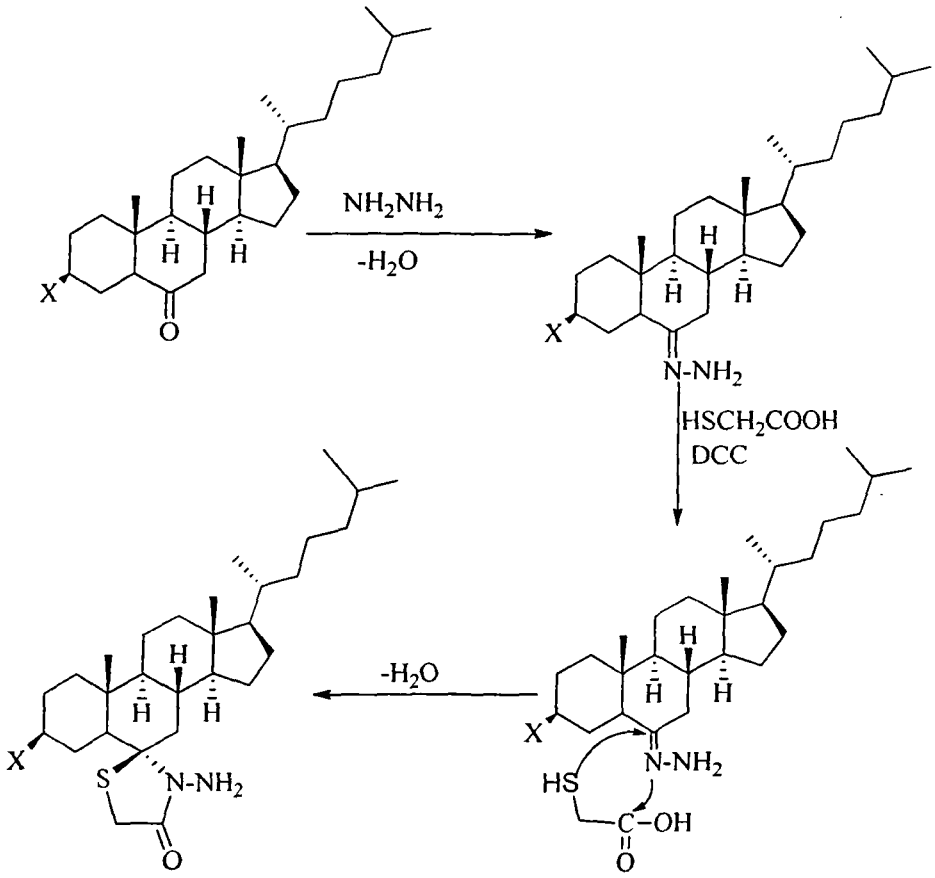
Characterization of compound 96 as 3β-chloro-5α-cholestan-(6R)-spiro-6,3'-amino-1', 3',4'-thiazolidinone:

The elemental analysis of compound **96** corresponded to the molecular formula $C_{29}H_{49}ClN_2OS$ (Beilstein positive). The IR data provided evidence for the formation of the expected structure. The compound showed intense band in the region of 3220 cm^{-1} due to NH_2 stretching vibrations. In addition, other important absorption bands at 1649, 1235, 776 and 683 cm^{-1} were attributed to C=O, C-N, C-Cl and C-S stretchings, respectively. Further evidence for the formation of compound **96** was well supported by its ^1H NMR and ^{13}C NMR spectra. The ^1H NMR spectrum of the compound exhibited two singlets at δ 8.13 (exchangeable with D_2O) for NH_2 and at 3.4 for methylene protones of thiazolidinone ring. A one-proton broad multiplet centered at δ 3.9 was assigned to C3-αH (axial, $W\,½$ = 17 Hz), Angular and side-chain methyl protons were observed at 1.19 (C10-CH_3), 0.75 (C13-CH_3), 0.91 and 0.85 for other methyl protons. ^{13}C NMR spectrum of the compound also supported the proposed structure and displayed characteristic signals at δ 174 for *C*=O, 50 for C_3, 39 for methylene carbon of thiazolidinone ring and 68 for C_6, in addition to the signals of

cholestane series. The mass spectrum was also in good agreement with its molecular formula which exhibited a strong molecular ion peak at *m/z* 508/510.

On account of the above evidences, the compound **95** can be suitably characterized as *3β-chloro-5α-cholestan-(6R)-spiro-6,3'-amino-1',3',4'-thiazolidinone.*

The tentative mechanism for the formation of thiazolidinones have been proposed in **scheme1**.



Scheme 1. Proposed mechanism of formation of 5α-cholestan-(6*R*)-spiro-6,3'-amino-1',3',4'-thiazolidinone derivatives

Stereochemistry

The stereoselectivity of steroidal thiazolidinone can be explained by considering that there is a considerable amount of steric hindrance to ring-closure from one side of the ring at C-6 which might be explained on the basis that during cyclization the thiazolidinone ring closes at C-6, by the attack of sulfur of mercaptoacetic acid moiety, preferentially from the front (β, axial) side so that the NNH_2 has an equatorial orientation (α) to avoid 1,3-diaxial interactions, giving minimum steric hindrance and maximum stability. This is further supported by the fact that during cyclization the nitrogen already attached to C-6 is moved towards the back (α, equatorial) side to reduce the steric hindrance, and leaving the front (β, axial) side for the attack of nucleophile to close the thiazolidinone ring at C-6. Therefore the only product of this reaction with *R* stereochemistry at C-6 was selectively obtained. The dreiding models also suggest the attack of sulfur from the β-side which pushes the nitrogen to the less hindered α-side. Hence the formulation of the compound as 6*R* is preferred over its isomer 6*S*. On the basis of these models it is suggested that the same should also be kinetically favorable.

Experimental

General

Melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were recorded on KBr pellets with Interspec 2020 FT-IR Spectrometer spectro Lab and values are given in cm^{-1}. ^1H and ^{13}C NMR spectra were run in $CDCl_3$ on a Bruker Avance II 500 NMR Spectrometer at 500 MHz and 125 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to the TMS (^1H NMR) and to the solvent signal (^{13}C NMR spectra). Mass spectra were recorded on a JEOL D-300 mass spectrometer. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapors to check the homogeneity as well as the progress of reaction. Sodium sulfate (anhydrous) was used as a drying agent

***5α-Cholestan-6-one* (91):**

6-Nitrocholest-5-ene (4 g) was dissolved in warm glacial acetic acid (80 mL) and zinc dust (8 g) was gradually added with shaking. The mixture was heated under reflux for 4 h and water (8 mL) was added during the course of reaction. Zinc dust (unreacted) was removed by filtration. To the filtrate, water was added till turbidity developed and it was allowed to stand overnight at room temperature. The solid material thus separated was filtered under suction and washed thoroughly with water in order to remove zinc acetate. The organic solid was air dried and then recrystallized from methanol (2.4 g), m.p. 96-98 °C (reported m.p. 98-100 °C).[57]

***3β-Acetoxy-5α-cholestan-6-one* (92):**

3β-Acetoxy-6-nitrocholest-5-ene (6.0 g) was dissolved in glacial acetic acid (120 mL) by warming the mixture and zinc dust (12.0 g) was added in small portions with shaking. The suspension was heated under reflux for 4 h and water (12 mL) was added at regular intervals during the course of reaction. The hot solution was filtered, cooled to room temperature and diluted with large excess of ice-cold water and extracted with diethyl ether. The ethereal solution was washed with water, $NaHCO_3$ solution (5%), again with water and dried over anhydrous sodium sulfate. Evaporation of solvents provided the acetoxy ketone as an oil which was crystallized from methanol (4.2 g) m.p. 128-129 °C (reported m.p. 127-128 °C).[58]

***3β-Chloro-5α-cholestan-6-one* (93):**

A mixture of 3β-chloro-6-nitrocholest-5-ene (8 g) and glacial acetic acid (160 mL) was heated just to get a clear solution. The zinc dust (16 g) was added gradually in small portions with constant shaking. The suspension was heated under reflux for 4 h and water (16 mL) was added at regular intervals during the course of reaction. The

hot solution was filtered and the filtrate was diluted with large excess of ice-cold water. The organic matter was extracted with diethyl ether and ethereal layer was washed successively with water, sodium bicarbonate solution (5 %) and again with water and dried over anhydrous sodium sulfate. Evaporation of the solvents furnished the ketone as an oil which was crystallized from methanol (5.8 g), m.p. 128-129 °C (reported m.p. 129 °C).[59]

***General procedure for the synthesis of steroidal thiazolidinone derivatives* (94-96):**

A mixture of steroidal ketone (**91-93**) (1 mmol), hydrazine hydrate (1 mmol) and mercaptoacetic acid (3.0 mmol) and DCC (1.2 mmol) were refluxed in benzene (15 mL) for 5-6 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the DCC was removed by filtration, and the solvent was removed under reduced pressure. The residue was taken in diethyl ether, washed with water and dried over anhydrous sodium sulfate. Removal of solvent gave the crude product which was recrystallized from methanol to obtain the corresponding pure product (**94-96**).

***5α-Cholestan-(6R)-spiro-6,3'-amino-1',3',4'-thiazolidinone* (94)**

Yield (78%); m.p. 128-130 °C; Anal. Calcd for $C_{29}H_{50}N_2OS$: C, 73.36; H, 10.61; N, 5.90; found; C, 73.34, H, 10.65, N, 5.92; IR (KBr) ν cm^{-1} 3215 (NH_2), 1645 (C=O), 1229 (C-N), 647 (C-S); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.11 (s, 1H, NH_2), 3.36 (s, 2H, CH_2), 1.19 (s, 3H, C10-CH_3), 0.85 (s, 3H, C13-CH_3), 0.91 and 0.85 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173, 67, 62, 56, 54, 50, 48, 43, 41, 40, 39, 38, 37, 35, 29, 28, 27, 26, 25, 24, 23, 22, 21, 21, 20, 20, 18, 19, 16; MS (EI): (*m/z*) 474 [$M^{+\cdot}$]

***3β-Acetoxy-5α-cholestan-(6R)-spiro-6,3'-amino-1',3',4'-thiazolidinone* (95)**

Yield (79%); m.p. 132-134 °C; Anal. Calcd for $C_{31}H_{52}N_2O_3S$: C, 69.88; H, 9.84; N, 5.26; found; C, 69.84, H, 9.45, N, 5.22; IR (KBr) ν cm^{-1} 3218 (NH_2), 1736 ($OCOCH_3$), 1648 (C=O), 1234 (C-N), 1234 (C-O), 680 (C-S); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.11 (s, 1H, NH_2), 4.7 (m, 1H, C3α-H, W ½=15 Hz, axial), 3.36 (s, 2H, CH_2), 2.03 (s, 3H, $OCOCH_3$), 1.19 (s, 3H, C10-CH_3), 0.85 (s, 3H, C13-CH_3), 0.91 and 0.85 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176, 172, 69, 62, 56, 54, 50, 49, 43, 41, 40, 39, 38, 37, 35, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 21, 20, 20, 18, 19, 16; MS (EI): (*m/z*) 532 [$M^{+\cdot}$]

3β-Chloro-5α-cholestan-(6R)-spiro-6,3'-amino-1',3',4'-thiazolidinone (96)

Yield (77%); m.p. 136-138 °C; (Beilstein positive); Anal. Calcd for $C_{29}H_{49}ClN_2OS$: C, 68.40; H, 9.70; N, 5.50; found; C, 68.37, H, 9.76, N, 5.54; IR (KBr) ν cm^{-1} 3220 (NH_2), 1649 (C=O), 1235 (C-N), 776 (C-Cl), 683 (C-S); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.13 (s, 1H, NH_2), 3.9 (m, 1H, C3α-H, W ½=17 Hz, axial), 3.4 (s, 2H, CH_2), 1.19 (s, 3H, C10-CH_3), 0.85 (s, 3H, C13-CH_3), 0.91 and 0.85 (other methyl protons); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174, 67, 68, 56, 54, 50, 49, 43, 41, 40, 39, 38, 37, 35, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 20, 18, 19, 16; MS (EI): (*m/z*) 508/510 [M^+]

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CHAPTER-5

Biological Evaluation

Of

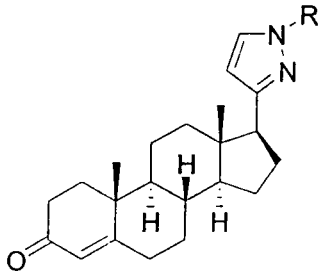
Newly Synthesized

Compounds

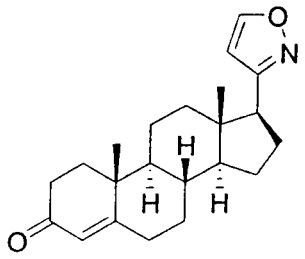
Theoretical

The global production of new drugs has increased in the last decades, and although many of the products have been beneficial for mankind, many of them are also toxic and can accumulate within organisms.[1] In the global drug market, steroid drugs rank second only after antibiotics.[2] Steroids have been a prime focus of research due to their fascinating structural framework and their excellent ability to penetrate cell membranes and bind to the nuclear and membrane receptors.[3] Even a small change in steroid moiety can elicit an extensive biological response. All these facts have attracted medicinal chemists and biochemists to explore these after modifying them suitably to induce various pharmacological properties.[4] Steroids represent constituents of biomembranes and hormones, fulfil protective functions, stimulate plant growth, etc. Many representatives of this group are widely used in medicine as essentials of anti-inflammatory, anabolic and contraceptive drugs[4] some of them are extremely toxic against tumor cells and show antimicrobial and other effects.[5] Heterocyclic analogs of steroids are of interest for the study of structure activity relationship.[6] They have an important practical value in the development of novel pharmacological agents for regulating and maintaining biochemical homeostasis in man and domestic animals. Attention to heterosteroid is caused by the fact that these compounds proved to be interesting from the biological activity point of view and moreover are convenient intermediates in the synthesis of numerous polyfunctional compounds.[7] The Heterocyclic analogs appended to the steroid nucleus were positioned on the α face and the β face of the steroid to test their ability to coordinate the heme iron of the P450 enzyme complex.[8] The position of the heterocycle with respect to the steroid skeleton was determined to be important for optimum affinity, and in general, compounds with the heterocycle attached to a trigonal centre at C-17, had the best affinity for C17(20) lyase.[9] The investigation of new modified steroid derivatives condensed with various heterocyclic rings has been given great attention.[10] The addition of heterocyclic rings to steroids often leads to a change of their physiological activity and the appearance of new interesting pharmacological and biological properties,[11] especially antiinflammatory,[12] antineoplastic[13] and antiandrogenic activity.[14] The treatment of infectious diseases still remains an important and challenging problem. The search of novel antimicrobial agents is field of current and growing interest and many compounds have been synthesized to this aim. Their clinical use has been limited by their relatively high risk of toxicity, bacterial resistance and/or pharmacokinetic deficiencies. A major research emphasis

to counter this growing problem is the development of antimicrobials structurally unrelated to the existing molecules. One possibility to achieve this goal is the combination of a steroid molecule with structural elements possessing appropriate biological activities[15]. The advantage of employing hydrophobic steroid units is their ability to interact with cell membranes and thus pave the way for biological activity of such hybrid molecules. Steroidal containing heterocycles were reported to be biologically active, here we are mentioning some reports by different workers for the biological evaluation of heterosteroids. Steroids heterocycles containing pyrazole (**1**), isoxazole (**2**), thiazole (**3**), pyrane (**4**) and pyridine ring (**5**) were reported to be active against some bacteria and fungi.[16]



(**1**)



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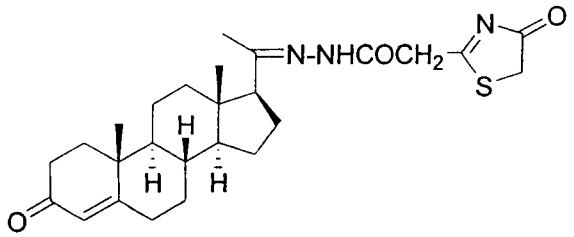

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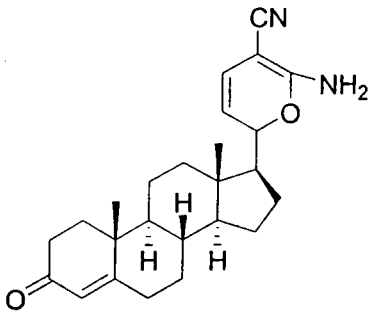
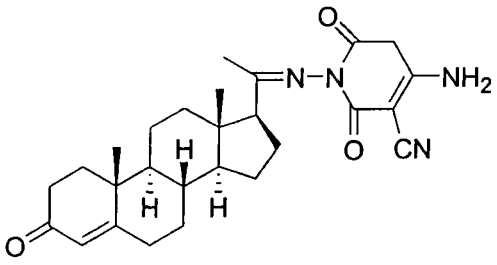
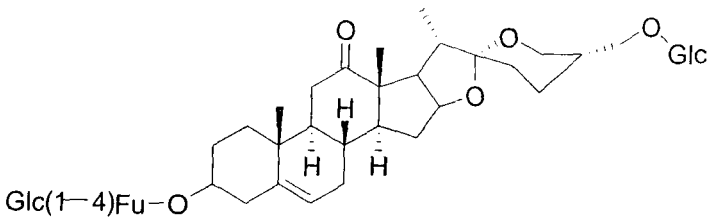


(**4**)

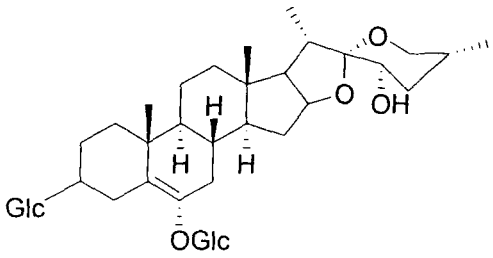


(**5**)

C27 steroidal saponins (**6**) and steroidal sapogenins (**7**) isolated from several monocotyledons plants were found to be effective against a class of fungi.[17]

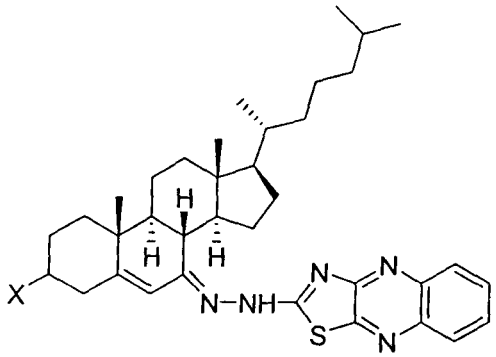


(6)



(7)

Saleem *et al.*[18] synthesized cholest-5-en-7-thiazolo[4, 5- *b*] quinoxaline-2-yl hydrazone derivatives (**8-10**) and reported them as promising antibacterial agents.



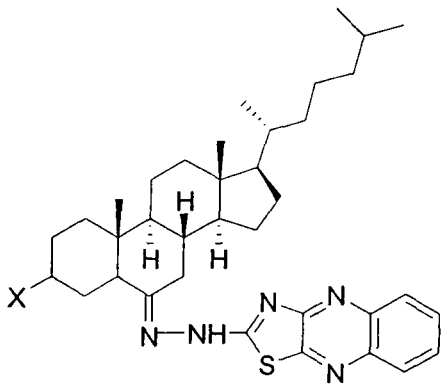
X

(8) H

(9) AcO

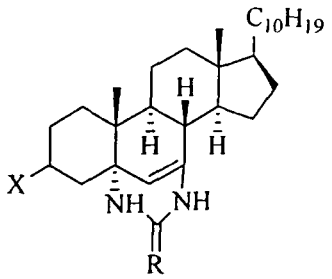
(10) Cl

Saleem *et al.* [19] reported the synthesis of different steroidal thiazolo quinooxaline (**11-13**) derivatives as antibacterial agents against *E. coli.* The antibacterial activities of these compounds were evaluated by disk diffusion method against the culture of *E. Coli* and the results were compared with the standard drug Amoxicillin.



	X
(11)	H
(12)	AcO
(13)	Cl

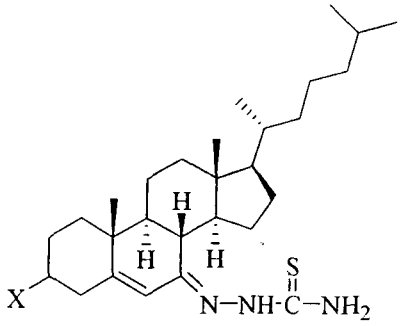
Stigmest-6-en-7,5 α thiourea (**14-15**) and stigmest-6-en-7,5 α urea (**16-17**) derivatives of steroids were found to be effective against a class of bacteria.[20]



	X	**R**
(14)	AcO	S
(15)	Cl	S
(16)	AcO	O
(17)	Cl	O

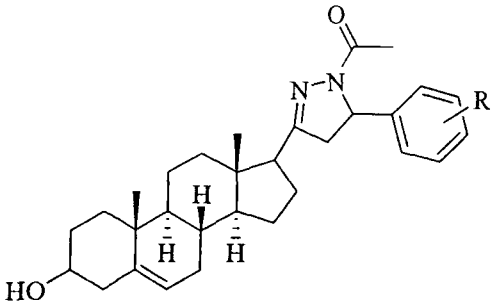
Several steroidal thiosemicarbazone derivatives (**18**) were also reported to be active against some bacteria. The antibacterial activity was first tested *in vitro* by disk

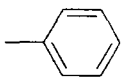
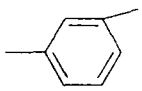
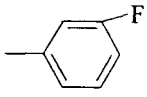
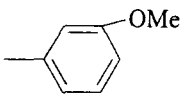
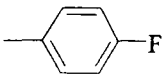
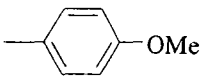
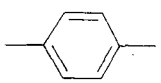
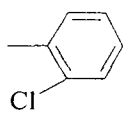
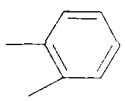
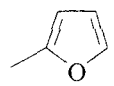
diffusion assay against two Gram-positive and two Gram-negative bacteria, and then the minimum inhibitory concentration (MIC) of compounds was determined.[21]



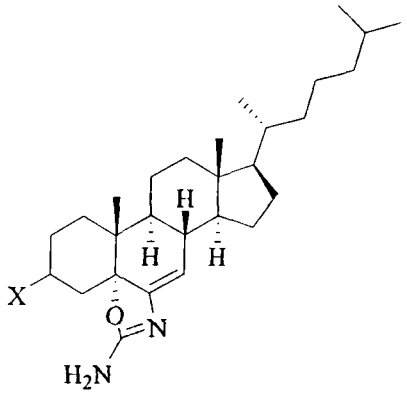
	X
(18)	H
(19)	AcO
(20)	Cl

Banday *et al.*[22] reported the synthesis of different steroidal pyrazoline **(21-30)** derivatives as anticancer agents.



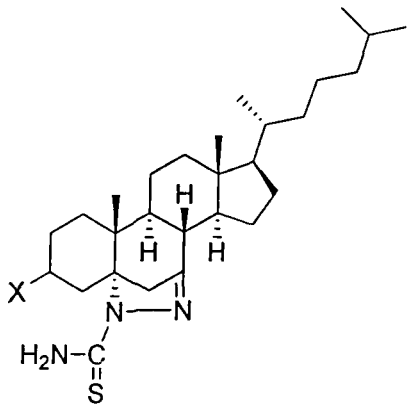
	R		R
(21)		**(26)**	
(22)		**(27)**	
(23)		**(28)**	
(24)		**(29)**	
(25)		**(30)**	

2′-amino-5a-cholest-6-eno [6, 5- *d*] oxazole derivatives (**31-33**) have found to be active against some class of microbial steroids.[23]



	X
(31)	H
(32)	AcO
(33)	Cl

Shamsuzzaman *et al.*[24] reported the synthesis of various steroidal pyrazolines (**34-36**) as antimicrobial agents.



	X
(34)	H
(35)	AcO
(36)	Cl

Discussion

Biological evaluation of steroidal pyrazoline derivatives 70-75

(Refer to Chapter-1 for their synthesis and characterization)

Rule of Five and bioactivity score

Calculated physicochemical properties of the synthesized compounds are shown in **Table 1**. The data showed one violation of Lipinski rules for compounds **70-73** due to a calculated Clog P value above the limit of 5 and two violations in compounds **74** and **75** due to the same reason and the molecular mass above 500 (**Table 1**). On the basis of the above results we can say that the synthesized compounds adhere to Lipinski's "Rule of Five" [25] and the exceptions to the Lipinski's rule of five are known and involve drugs that are transported across membranes by carrier proteins, such as antibiotic erythromycin.[26]

Table 1. Calculated physicochemical properties of steroidal pyrazolines 70-75.

Comp.	Mwt	ClogP	HBD	HBA	TPSA	No. violations
70	398.67	10.68	1	1	22.31	1
71	456.70	7.45	1	3	22.31	1
72	433.11	8.578	1	1	43.61	1
73	474.76	11.526	1	1	21.31	1
74	532.80	10.38	1	3	21.31	2
75	509.21	11.51	1	1	42.31	2

Clog P; expressed as the logarithm of its partition coefficient between n-octanol and water $\log(c_{octanol}/c_{water})$

HBD; Hydrogen bond donor (expressed as the sum of OH and NH)

HBA; Hydrogen bond acceptor (expressed as the sum of O and N atoms)

TPSA; Topological polar surface area (defined as a sum of surfaces of polar atoms in a molecule)

The bioactivity score of the compounds **70-75** was also calculated for six criteria, GPCR ligand activity, ion channel modulation, kinase inhibition activity, protease inhibitor, enzyme inhibitor and nuclear receptor ligand activity. As we know for organic molecules if the bioactivity score is more than 0.00 then the compound is active but if it is between -0.50 to 0.00 then the compound is moderately active and if the compound has less than -0.50 then it is inactive compound. As we can see in **Table 2** our synthesized compounds showed good bioactivity score.

Table 2. Bioactivity score of steroidal pyrazolines **70-75.**

Comp.	GPCR ligand	Ion channel	Modulator Kinase	Protease inhibitor	Nuclear Receptor ligand	Enzyme inhibitor
70	-0.21	-0.21	-0.73	-0.20	0.23	-0.04
71	-0.20	-0.10	-0.72	-0.08	0.23	0.03
72	-0.20	-0.25	-0.75	-0.25	0.17	-0.04
73	-0.15	-0.20	-0.70	-0.17	0.14	0.01
74	-0.15	-0.22	-0.70	-0.08	0.13	0.05
75	-0.14	-0.24	-0.71	-0.12	0.09	0.01

Anticancer activity

Steroidal pyrazolines (**70-75**) were evaluated for cytotoxicity in a panel of selective human cancer cell lines using the MTT assay. The panel of cancer cells encompassed HepG2 (from hepatocellular carcinoma), A549 (from lung adenocarcinoma epithelium), SW480 (from colon adenocarcinoma), HeLa (from cervical carcinoma) and HL60 from (promyelocytic leukaemia). Doxorubicin and Cytarabine were used as cytotoxic drugs of reference. It's clear from IC_{50} values (**Table 3**) that all the compounds showed impressive activity against tested cancer cell lines. Compound **70** was found to be potential inhibitor of cell death against all cancer cell lines except HL60. Compound **71** was specific to HepG2 (IC_{50}= 17.04) while compounds **72-75**, showed marked inhibitory activity against HL60 (IC_{50}= 14.29), SW480 (IC_{50}=12.17), HepG2 (IC_{50}=19.24) and SW480 (IC_{50}=16.25) respectively. To confirm the cytotoxicity the synthesized compounds **70-75** were also tested with non-cancer cell lines **PMBC** during which none of the compounds were found toxic, all the compounds showed IC_{50}> 60 μmol L^{-1}. This also suggests that the steroidal pyrazoline derivatives can be used specifically for the treatment of cancer cells without showing toxicity to the non-cancer cells (**Table 3**).

Table 3. Anticancer activity of steroidal pyrazolines **70-75**.

Comp	SW480	A549	HepG2	HeLa	HL60	PMBC
70	11.97±0.2	11.05±0.2	25.03±0.5	16.32±0.3	>50	65
71	28.01±0.4	28.21±0.3	17.04±0.2	>50	23.15±0.2	64
72	26.21±0.3	33.52±0.6	38.14±0.2	22.13±0.5	14.29±0.6	65
73	12.17±0.2	13.43±0.2	25.03±0.5	16.32±0.3	32.42±0.2	63
74	28.01±0.4	28.21±0.3	19.24±0.2	20.43±0.2	23.15±0.2	69
75	16.25±0.4	30.32±0.6	28.24±0.2	22.03±0.5	17.10±0.6	65
Doxorubicin	10.9±0.4	15.01±0.3	13.5±0.3	13.52±0.3	9.52±0.2	
Cytarabine	16.05±0.2	14.12±0.6	13.04±0.4	14.32±0.2	10.09±0.2	

Antimicrobial Activity

The synthesized compounds **70-75** were screened for their *in vitro* antimicrobial activities against Gram-positive and Gram-negative bacterial strains and were found to possess activities against the bacteria listed in **Tables 4** and 5. All of the compounds present excellent activities against Gram-positive bacteria, exhibiting MIC values of 25-100 μg/mL. On the other hand, all the derivatives possess moderate to excellent antimicrobial activities against Gram-negative strains. Among the synthesized compounds it was clear that compound **70** showed very good antibacterial activity nearly equivalent to that of standard drug Ciprofloxacin. For assaying antifungal activity, different fungal strains like *Candida albicans*, *Aspergillus fumigatus*, *Pencillium marneffei* and *Trichophyton mentagrophytes* were chosen. The antifungal screening data showed moderate to good fungal inhibition (**Tables 6** and 7). Among the screened compounds, compound **70** and **73** were found to have good zones of inhibition. The MFC of all the compounds was two or three folds higher than the corresponding MIC results.

Table 4. Antibacterial activity (zones of inhibition) of steroidal pyrazolines **70-75.**

Comp.	Diameter of zone of inhibition (mm)				
	Gram positive bacteria			Gram negative bacteria	
	S. Pyogenes	*MRSA**	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
70	16.9±0.3	18.9±0.4	19.2±0.3	15.8±0.3	16.3±0.6
71	15.7±0.2	15.2±0.5	17.1±0.3	15.2±0.4	15.1±0.2
72	14.1±0.5	14.7±0.2	15.9±0.6	13.1±0.6	13.9±0.4
73	15.6±0.3	16.9±0.4	15.2±0.3	12.8±0.3	14.3±0.6
74	13.6±0.3	12.2±0.2	14.3±0.3	14.2±0.4	9.1±0.2
75	13.1±0.5	15.7±0.2	14.9±0.6	13.1±0.6	12.9±0.4
Standard	23.0±0.2	22.0±0.2	32.0±0.3	19.0±0.2	27.0±0.2
DMSO	-	-	-	-	-

Positive control (standard); Ciprofloxacin and negative control (DMSO) measured by the Halo Zone Test (Unit, mm); * Methicillin resistant *Staphylococcus aureus* (MRSA +Ve).

Table 5. MIC and MBC results of steroidal pyrazolines **70-75** against bacterial strains.

Comp.	Gram positive bacteria				Gram negative bacteria					
	S.Pyogenes		*MRSA**		*P. aeruginosa*		*K. pneumonia*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
70	50	100	50	100	50	100	50	>100	50	>100
71	50	100	50	>100	50	50	100	>100	100	>100
72	25	100	12	50	25	50	12	50	25	>100
73	50	100	50	100	50	100	50	>100	50	>100
74	50	100	50	>100	50	50	100	>100	100	>100
75	50	100	25	50	50	100	50	>100	50	>100
Standard	12.5	12.5	6.25	12.5	12.5	25	6.25	25	6.25	12.5

CA; *Candida albicans*, AF; *Aspergillus fumigatus*, TM; *Trichophyton mentagrophytes*, PM; *Penicillium marneffei*. Positive control (standard); Fluconazole and negative control (DMSO) measured.

Table 7. MIC and MFC of of steroidal pyrazolines **70-75** against fungal strains.

Comp.	CA		AF		TM		PM	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
70	12	50	25	100	50	100	12	100
71	25	100	50	100	100	100	50	100
72	50	100	50	100	100	>100	100	>100
73	12	50	12	100	50	100	25	100
74	25	100	50	100	50	100	50	100
75	50	100	50	100	100	>100	100	> 00
Standard	6.25	25	12.5	12.5	6.25	25	12.5	25

(Standard); Fluconazole; CA; *Candida albicans*, AF; *Aspergillus fumigatus*, TM; *Trichophyton mentagrophytes*, PM; *Penicillium marneffei*. MIC (μg/ml) = minimum inhibitory concentration, i.e the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (μg/ml) = minimum fungicidal concentration, i.e., the lowest concentration of the compound for killing the fungus completely.

In vitro antioxidant activity

The *in vitro* antioxidant activity and scavenging effects of steroidal pyrazolines **70-75** were evaluated by using different reactive species assay containing DPPH radical scavenging activity. The free radical scavenging activity of all the synthesized compounds were evaluated through their ability to quench the DPPH' using ascorbic acid as reference. The potencies for the antioxidant activity of the synthesized compounds to the reference drug are shown in **Table 8**. In general all the synthesized compounds were less potent than the reference. Among the synthesized compounds, compounds **70** and **72** exhibited a slightly better antioxidant activity than the other compounds.

Table 8. The antioxidant activity data of steroidal pyrazolines **70-75**[a].

	% inhibition			
Comp.	**25 µg/mL**	**50 µg/mL**	**75 µg/mL**	**100 µg/mL**
70	15.8±0.5	23.7±0.3	15.6±0.6	24.5±0.4
71	14.8±0.7	11.6±0.5	16.6±0.8	21.9±0.9
72	14.2±0.2	18.3±0.4	22.6±0.2	25.5±0.3
73	19.2±0.2	21.1±0.3	11.7±0.3	14.5±0.4
74	13.3±0.4	17.5±0.4	16.6±0.8	12.9±0.9
75	18.1±0.2	19.7±0.2	12.6±0.2	15.8±0.2
Standard	36.0±0.3	37.0±0.2	44.0±0.3	50.0±0.5
control[b]	-	-	-	-

[a]Values represent the mean ± standard error mean (SEM) of three experiments.
[b]No inhibition, standard: ascorbic acid.

Biological evaluation of steroidal pyranone derivatives 105-110

(Refer to Chapter-2 for their synthesis and characterization)

Rule of Five and bioactivity score

The synthesized steroidal pyranones **105, 107** and **108** showed one violation of Lipinski rules for compounds due to a calculated Clog P value above the limit of 5 and two violations in the rest of compounds (**106, 109** and **110**) due to the same reason and the molecular mass above 500 (**Table 9**). On the basis of these results we can say that the synthesized compounds adhere to Lipinski's Rule.[23]

Table 9. Calculated physicochemical properties of steroidal pyranones **105-110**.

Comp.	Mwt	ClogP	HBD	HBA	TPSA	No. violations
105	461.12	8.518	0	2	20.31	1
106	534.19	8.544	0	4	20.31	2
107	495.56	9.518	0	2	46.61	1
108	468.71	9.092	0	3	22.32	1
109	526.7	7.951	0	5	22.32	2
110	503.16	8.925	0	3	48.61	2

Clog P; expressed as the logarithm of its partition coefficient between n-octanol and water $\log(c_{octanol}/c_{water})$

HBD; Hydrogen bond donor (expressed as the sum of OH and NH)

HBA; Hydrogen bond acceptor (expressed as the sum of O and N atoms)

TPSA; Topological polar surface area (defined as a sum of surfaces of polar atoms in a molecule)

The bioactivity score of the compounds (**105-110**) was also calculated for six criteria, GPCR ligand activity, ion channel modulation, kinase inhibition activity, protease inhibitor, enzyme inhibitor and nuclear receptor ligand activity **Table 10**. The results show that these compounds have good bioactivity score.

Table 10. Bioactivity score of steroidal pyranones **105-110.**

Comp.	GPCR ligand	Ion channel	Modulator Kinase	Protease inhibitor	Nuclear receptor ligand	Enzyme inhibitor
105	0.03	-0.01	-0.57	0.13	0.34	0.26
106	0.03	-0.03	-0.56	0.16	0.35	0.32
107	0.04	-0.05	-0.59	0.07	0.28	0.27
108	-0.07	-0.03	-0.72	0.04	0.28	0.24
109	-0.05	-0.10	-0.70	0.08	0.30	0.29
110	-0.07	-0.09	-0.77	-0.00	0.22	0.21

Anticancer activity

Steroidal pyranones were evaluated for cytotoxicity in a panel of selective human cancer cells using the MTT assay (**Table 11**). The panel of cancer cells encompassed HepG2 (from hepatocellular carcinoma), A549 (from lung adenocarcinoma epithelium), SW480 (from colon adenocarcinoma), HeLa (from cervical carcinoma) and HL-60 from (promyelocytic leukaemia). Doxorubicin and Cytarabine were used as cytotoxic drugs of reference. Anticancer potency of the compounds was indicated by IC_{50} values that were calculated by linear regression analysis of the concentration-response curves obtained for each compound. Compound **105** was found to be most active among all the synthesized compounds against almost all the cancer cell lines while compounds **106, 108, 109** and **110** were found more potential to HeLa (IC_{50}=16.32), HeLa (IC_{50}=16.32), HepG2 (IC_{50}=19.24) and HepG2 (IC_{50}=18.14), respectively.

To confirm the cytotoxicity, the synthesized compounds were also tested with non-cancer cell lines **PMBC** during which none of the synthesized compounds were found toxic, all the compounds showed IC_{50}> 60 $\mu molL^{-1}$.

Table 11. Anticancer activity of steroidal pyranones **105-110**.

Comp	SW480	A549	HepG2	HeL	HL60	PMBC
105	18.27±0.2	19.15±0.2	15.03±0.5	16.32±0.3	19.61±0.	69
106	29.21±0.5	19.11±0.8	37.24±0.2	16.32±0.3	43.45±0.2	68
107	26.61±0.6	23.22±0.2	27.34±0.5	22.23±0.6	24.19±0.7	64
108	42.27±0.2	33.43±0.	25.13±0.5	16.32±0.3	32.42±0.2	63
109	38.01±0.2	29.11±0	19.24±0.2	22.43±0.2	23.45±0.2	69
110	25.15±0.4	27.22±0.6	18.14±0.5	29.03±0.8	27.10±0.	68
Doxorubicin	10.9±0.4	13.5±0.3	14.12±0.6	13.52±0.3	9.52±0.2	-
Cytarabine	15.01±0.3	16.05±0.2	13.04±0.4	14.32±0.2	10.09±0.2	-

Antimicrobial Activity

The synthesized compounds **105-110** were also screened for their *in vitro* antimicrobial activities against Gram-positive and Gram-negative bacterial strains and were found to possess activities against the microorganisms listed in **Tables 12** and **13**. All of the compounds present excellent activities against Gram-positive bacteria, exhibiting MIC values of 25-100 μg/mL. On the other hand, all the derivatives

possess moderate to excellent antimicrobial activities against Gram-negative strains. Among the synthesized compounds it was clear that compound **107** showed very good antibacterial activity nearly equivalent to that of standard drug Ciprofloxacin. For assaying antifungal activity, different fungal strains like *Candida albicans*, *Asperergillus fumigatus*, *Pencillium marneffei* and *Trichophyton mentagrophytes* were chosen. The antifungal screening data showed moderate to good fungal inhibition (**Tables 14** and **15**). Among the screened compounds, **107** and **109** were found to have good zones of inhibition. The MFC of all the compounds was two or three folds higher than the corresponding MIC results.

Table 12. Antibacterial activity (zones of inhibition) of steroidal pyranones **105-110.**

Diameter of zone of inhibition (mm)					
Comp.	***Gram positive bacteria***		***Gram negative bacteria***		
	S. Pyogenes	***MRSA****	***P. aeruginosa***	***K. pneumoniae***	***E. coli***
105	13.5±0.5	18.1±0.4	19.2±0.3	15.8±0.3	16.3±0.6
106	12.3±0.2	15.2±0.5	17.1±0.3	15.2±0.4	15.1±0.2
107	17.1±0.5	13.7±0.2	13.9±0.6	10.1±0.6	13.9±0.4
108	17.2±0.3	15.9±0.4	18.2±0.3	11.3±0.	14.3±0.5
109	12.6±0.3	11.2±0.2	11.3±0.3	12.1±0.4	19.1±0.2
110	18.2±0.9	18.2±0.1	17.9±0.6	17.1±0.2	16.9±0.2
Standard	23.0±0.2	22.0±0.2	32.0±0.3	19.0±0.2	27.0±0.2
DMSO	-	-	-	-	-

Positive control (standard); Ciprofloxacin and negative control (DMSO) measured by the Halo Zone Test (Unit, mm); * Methicillin resistant *Staphylococcus aureus* (MRSA +Ve)

Table 13. MIC and MBC results of steroidal pyranones **105-110** against bacterial strains.

Comp.	Gram positive bacteria				Gram negative bacteria					
	S. Pyogenes		*MRSA**		*P. aeruginosa*		*K. pneumonia*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
105	50	100	50	100	50	100	50	>100	50	>100
106	50	100	50	>100	50	50	100	>100	100	>100
107	25	100	12	50	25	50	12	50	25	>100
108	50	100	50	100	50	100	50	>100	50	>100
109	50	100	50	>100	50	50	100	>100	100	>100
110	50	100	25	50	50	100	50	>100	50	>100
Standard	12.5	12.5	6.25	12.5	12.5	25	6.25	25	6.25	12.5

(Standard); Ciprofloxacin; MIC (µg/ml) = minimum inhibitory concentration, i.e the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (µg/ml) = minimum bacterial concentration, i.e., the lowest concentration of the compound for killing the bacteria completely; * Methicillin resistant *Staphylococcus aureus* (MRSA +Ve).

Table 14. Antifungal activity of steroidal pyranones **105-110**.

	Diameter of zone of inhibition (mm)			
Comp.	**CA**	**AF**	**TM**	**PM**
105	29.2±0.2	22.7±0.1	20.6±0.6	19.5±0.4
106	22.1±0.2	21.7±0.3	14.6±0.2	16.5±0.1
107	26.4±0.4	19.9±0.6	18.9±0.5	11.8±0.2
108	25.6±0.2	21.7±0.3	14.6±0.2	14.5±0.4
109	22.8±0.6	22.5±0.3	12.6±0.1	13.5±0.1
110	20.8±0.8	18.9±0.6	18.9±0.2	11.4±0.2
Standard	30.0±0.2	27.0±0.2	24.0±0.3	20.0±0.5
DMSO	-	-	-	-

CA; *Candida albicans*, AF; *Aspergillus fumigatus*, TM; *Trichophyton mentagrophytes*, PM; *Penicillium marneffei*. Positive control (standard); Fluconazole and negative control (DMSO) measured.

Table 15. MIC and MFC of of steroidal pyranones **105-110** against fungal strains.

Comp.	CA		AF		TM		PM	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
105	12	50	25	100	50	100	12	100
106	25	100	50	100	100	100	50	100
107	25	100	50	100	10	50	100	>100
108	12	50	12	100	50	100	25	100
109	25	100	50	100	50	100	50	100
110	50	100	50	100	100	>100	100	>100
Standard	6.25	25	12.5	12.5	6.25	25	12.5	25

(Standard); Fluconazole; CA; *Candida albicans*, AF; *Aspergillus fumigatus*, TM; *Trichophyton mentagrophytes,* PM; *Penicillium marneffei.* MIC (µg/ml) = minimum inhibitory concentration, i.e the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (µg/ml) = minimum fungicidal concentration, i.e., the lowest concentration of the compound for killing the fungus completely.

In vitro antioxidant activity

The *in vitro* antioxidant activity and scavenging effects of steroidal pyranone derivatives were evaluated by using different reactive species assay containing DPPH radical scavenging activity. The free radical scavenging activity of all the synthesized compounds were evaluated through their ability to quench the $DPPH^{\bullet}$ using ascorbic acid as reference. The compounds **108** exhibited a slightly better antioxidant activity than the rest of compounds as shown in **Table 16.**

Table 16. The antioxidant activity data of steroidal pyranones **105-110**[a].

	% inhibition			
Comp.	**25 µg/mL**	**50 µg/mL**	**75 µg/mL**	**100 µg/mL**
105	17.1±0.4	22.5±0.3	25.6±0.3	21.5±0.4
106	15.8±0.2	21.6±0.5	19.6±0.5	20.9±0.9
107	18.2±0.7	18.2±0.4	21.6±0.1	15.5±0.3
108	28.2±0.6	20.1±0.3	31.7±0.2	34.5±0.4
109	15.3±0.2	19.5±0.4	16.6±0.7	22.9±0.9
110	19.2±0.5	25.7±0.2	12.6±0.5	25.8±0.2
Standard	36.0±0.3	37.0±0.2	44.0±0.3	50.0±0.5
Control[b]	-	-	-	-

[a]Values represent the mean ± standard error mean (SEM) of three experiments.
[b]No inhibition, standard: ascorbic acid.

Biological evaluation of steroidal pyrazolone derivatives 83-85

(Refer to Chapter-3 for their synthesis and characterization)

Rule of Five and bioactivity score

The compound **83** showed one violation of Lipinski rules due to a calculated Clog P value above the limit of 5 and two violations in compounds **84** and **85** due to the same reason and the molecular mass above 500. On the basis of these results we can say that the synthesized compounds adhere to Lipinski's "Rule of Five" (**Table 17**).[23]

Table 17. Calculated physicochemical properties of steroidal pyrazolone derivatives **83-85**

Comp.	Mwt	ClogP	HBD	HBA	TPSA	No. violations
83	467.742	7.979	3	4	71.777	1
84	525.778	7.298	3	6	98.082	2
85	502.187	7.832	3	4	71.777	2

Clog P; expressed as the logarithm of its partition coefficient between n-octanol and water $\log(c_{octanol}/c_{water})$

HBD; Hydrogen bond donor (expressed as the sum of OH and NH)

HBA; Hydrogen bond acceptor (expressed as the sum of O and N atoms)

TPSA; Topological polar surface area (defined as a sum of surfaces of polar atoms in a molecule)

The bioactivity score of compounds **83-85** was also calculated for GPCR ligand activity, ion channel modulation, kinase inhibition activity, protease inhibitor, enzyme inhibitor and nuclear receptor ligand activity. As we know for organic molecules if the bioactivity score is more than 0.00 then the compound is active but if it is between -0.50 to 0.00 the compound is moderately active and if the compound has less than -0.50 it is considered as inactive compound. Therefore the result (**Table 18**) showed that compounds **83-85** had good bioactivity score.

Table 18. Bioactivity score of steroidal pyrazolones **83-85.**

Comp.	GPCR ligand	Ion channel	Modulator Kinase	Protease inhibitor	Nuclear receptor ligand	Enzyme inhibitor
83	0.00	0.06	-0.12	0.09	-0.05	0.27
84	-0.00	-0.08	-0.12	-0.01	-0.07	0.19
85	-0.04	-0.02	-0.05	0.0	-0.05	0.21

Anticancer activity

Steroidal pyrazolones (**84-86**) were evaluated for anticancer activity against selective human cancer cells using the MTT assay (**Table 19**). The panel of cancer cells encompassed HepG2, A549, SW480, HeLa and HL-60. Doxorubicin and Cytarabine were used as cytotoxic drugs of reference. The compound **84** showed potential activity with minimum IC_{50}=11.67 (SW480), 16.32 (HeLa), 19.61 (A549) μmol L^{-1} while compound **85** showed minimum IC_{50}=17.04 (HepG2) and 18.01 (SW480) μmol L^{-1}.

Table 19. Anticancer activity of steroidal pyrazolone derivatives **83-85** against cancer and non-cancer cells.

Comp.	SW480	A549	HepG2	HeLa	HL60	PMBC
83	26.21±0.3	33.52±0.6	38.14±0.2	22.03±0.5	14.09±0.6	64
84	11.67±0.2	19.05±0.2	25.03±0.5	16.32±0.3	>50	69
85	18.01±0.4	29.21±0.3	17.04±0.2	>50	23.15±0.2	67
Doxorubicin	10.9±0.4	13.5±0.3	11.52±0.6	12.52±0.3	9.52±0.2	-
Cytarabine	15.01±0.3	16.05±0.2	13.04±0.4	14.32±0.2	10.09±0.2	-

To confirm their cytotoxicity the compounds **83-85** were also tested with non-cancer cell lines **PMBC** during which none of these compounds were found toxic, all the compounds showed IC_{50}> 60 μmol L^{-1}. This also suggested that the steroidal pyrazolone derivatives can be used specifically for the treatment of cancer cells without showing toxicity to the normal cells (**Table 19**).

Antimicrobial Activity

The compounds **83-85** were evaluated for their *in vitro* antimicrobial activities against Gram-positive and Gram-negative bacterial strains and were found to possess activities against the microorganisms listed in **Tables 20** and **21**. All of the compounds presented excellent activities against Gram-positive bacteria, exhibiting MIC values of 25-100 μg/mL suggesting that the presence of amino group may lead to the biologically activity of the compounds. In addition to this, all the derivatives also show moderate to excellent antimicrobial activity against Gram-negative strains.. Among these compounds it was clear that compound **83** showed very good antibacterial activity nearly equivalent to that of standard drug Ciprofloxacin.

Table 20. Antibacterial activity (zones of inhibition) of steroidal pyrazolones **83-85**.

Diameter of zone of inhibition (mm).					
Comp.	**Gram positive bacteria**		**Gram negative bacteria**		
	S. Pyogenes	***MRSA****	***P. aeruginosa***	***K. pneumoniae***	***E. coli***
83	16.9±0.3	15.9±0.4	19.2±0.3	15.8±0.3	16.3±0.6
84	15.7±0.2	15.2±0.5	17.1±0.3	15.2±0.4	15.1±0.2
85	14.1±0.5	14.7±0.2	15.9±0.6	13.1±0.6	13.9±0.4
Standard	23.0±0.2	22.0±0.2	32.0±0.3	19.0±0.2	27.0±0.2
DMSO	-	-	-	-	-

Positive control (standard); Ciprofloxacin and negative control (DMSO) measured by the Halo Zone Test (Unit, mm); * Methicillin resistant *Staphylococcus aureus* (MRSA +Ve).

Table 21. MIC and MBC results of steroidal pyrazolones **83-85** against bacterial strains.

Comp.	**Gram positive bacteria**				**Gram negative bacteria**					
	S. Pyogenes		***MRSA****		***P.aeruginosa***		***K.pneumonia***		***E. coli***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
83	50	100	25	50	50	100	50	>100	50	>100
84	50	100	50	100	50	100	50	>100	50	>100
85	50	100	50	>100	50	50	100	>100	100	>100
Standard	12.5	12.5	6.25	12.5	12.5	25	6.25	25	6.25	12.5

(Standard); Ciprofloxacin; MIC (μg/ml) = minimum inhibitory concentration, i.e the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (μg/ml) = minimum bacterial concentration, i.e., the lowest concentration of the compound for killing the bacteria completely; * Methicillin resistant *Staphylococcus aureus* (MRSA +Ve).

For assaying antifungal activity, different fungal strains like *Candida albicans*, *Asperergillus fumigatus*, *Pencillium marneffei* and *Trichophyton mentagrophytes* were chosen. The antifungal screening data showed moderate to good fungal inhibition (**Tables 22** and **23**).

Table 22. Antifungal activity of steroidal pyrazolones **83-85**.

	Diameter of zone of inhibition (mm)			
Comp.	**CA**	**AF**	**TM**	**PM**
83	27.8±0.2	22.7±0.3	18.6±0.6	16.5±0.4
84	25.8±0.2	20.7±0.3	17.6±0.6	14.5±0.4
85	20.9±0.4	14.9±0.3	12.8±0.5	12.8±0.5
Standard	30.0±0.2	27.0±0.2	24.0±0.3	20.0±0.5
DMSO	-	-	-	-

CA; *Candida albicans*, AF; *Aspergillus fumigatus*, TM; *Trichophyton mentagrophytes*, PM; *Penicillium marneffei*. Positive control (standard); Fluconazole and negative control (DMSO) measured.

Among the screened compounds, **84** and **85** were found to have good zones of inhibition. The compound **83** showed maximum inhibition against *C. albicans* and *Trichophyton mentagrophytes* strains, while compound **83** was more effective by showing maximum inhibition against *C. albicans*, *Aspergillus fumigatus* and *Penicillium marneffei* strain. The MFC of all the compounds was two or three folds higher than the corresponding MIC results.

Table 23. MIC and MFC of steroidal pyrazolones **83-85** against fungal strains.

Comp.	**CA**		**AF**		**TM**		**PM**	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
83	25	50	25	100	50	100	25	100
84	25	100	50	100	50	100	50	100
85	50	100	50	100	100	>100	100	>100
Standard	6.25	25	12.5	12.5	6.25	25	12.5	25

(Standard); Fluconazole; CA; *Candida albicans*, AF; *Aspergillus fumigatus*, TM; *Trichophyton mentagrophytes*, PM; *Penicillium marneffei*. MIC (μg/ml) = minimum inhibitory concentration, i.e the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (μg/ml) = minimum fungicidal concentration, i.e., the lowest concentration of the compound for killing the fungus completely.

The deduced patterns of antimicrobial activity of the newly synthesized steroidal pyrazolones are in the following order: antifungal > antibacterial.

In vitro antioxidant activity

The *in vitro* antioxidant activity and scavenging effects of steroidal pyrazolone derivatives **83-85** were evaluated by using different reactive species assay containing DPPH radical scavenging activity. The free radical scavenging activity of these compounds were evaluated through their ability to quench the DPPH• using ascorbic acid as reference. The potencies for the antioxidant activity of the compounds to the reference drug are shown in **Table 24**

Table 24. The antioxidant activity data of steroidal pyrazolones **83-85** [a].

	% inhibition			
Comp.	**25 µg/mL**	**50 µg/mL**	**75 µg/mL**	**100 µg/mL**
83	16.1±0.2	21.5±0.3	24.6±0.5	21.3±0.2
84	14.2±0.2	18.9±0.2	14.6±0.2	21.9±0.8
85	18.2±0.5	22.2±0.5	10.6±0.5	24.8±0.2
Standard	36.0±0.3	37.0±0.2	44.0±0.3	50.0±0.5
Control[b]	-	-	-	-

[a]Values represent the mean ± standard error mean (SEM) of three experiments.
[b]No inhibition, standard: ascorbic acid.

In general, all the synthesized compounds (**83-85**) were less potent than the reference.

Molecular Docking

Molecular docking of compounds 83-85 with S12 protein (PDB ID: 1FJG)

In order to know the exact interactions of the compounds **83-85** docking studies were carried out. **Figures 1** depicts the comparison of binding of these compounds with the Ciprofloxacin binding site. Although the compounds **83**, **84** and **85** had no hydrogen bonds with the S12 protein but have hydrophobic interactions with the S12 protein.

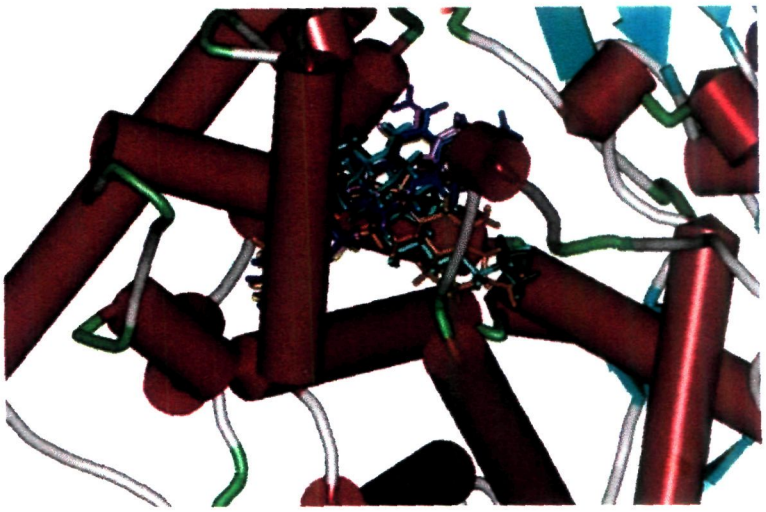


Figure 1 (a). All the three molecules (**83-85**) docked in the binding site of S12 protein.

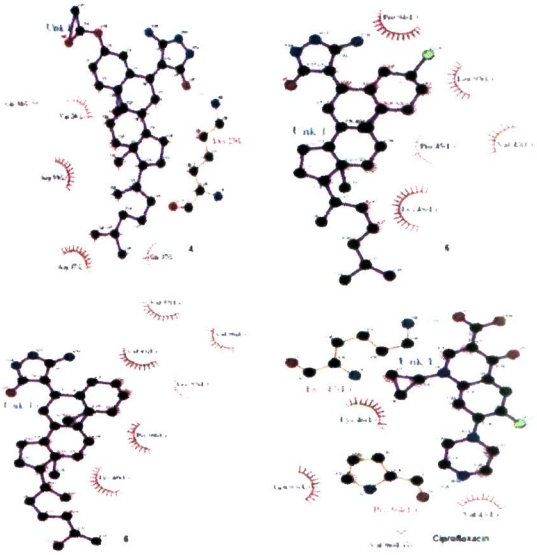


Figure 1 (b). (Ligplot) The interaction plot of all the three molecules (**83-85**) with the binding site of S12 protein

The top score pose was selected for each compound and their results are shown in **Table 25** and compared with Ciprofloxacin, which was redocked with the target protein using the same protocol. Among these three compounds, compound **83** showed strong molecular interactions within active site of S12 with 56.78 GOLD fitness score as compared to other compounds **84**, **85** and reference drugs having 54.45, 52.62 and 55.45 fitness scores, respectively. Compound **83** was also found to have highest number of hydrophobic contacts (twenty six) as compared to other novel compounds and reference drug Ciprofloxacin.

Table 25. The ligand molecules with number of molecular interactions and the scores for strength of binding with S 12 protein of *E. coli.*

Comp.	GOLD fitness score	Residues involved in Hydrogen bonding	Residues involved in Hydrophobic interaction	H-bond Range (Å)	Hydrophobic Interaction Range (Å)
83	56.78	NA	Val43,Lys46,Val55 ,Leu93,Pro94,Val9 6	NA	3.24-3.90
84	54.45	NA	Val43, Pro45, Lys46,Pro94	NA	3.17-3.90
85	52.62	NA	Val43,Lys46,Val55 ,Leu93, Pro94,Val96	NA	3.12-3.87
Ciprofloxacin	55.45	Lys47, Pro94	Val43, Lys46, Lys47,Leu93, Val96	2.6-2.9	3.2-3.80

It was evident from **Figures 1** that for each compound, the binding site and hydrogen-bonding interactions was found varied. It was interesting to observe that even though the core structure of all the compounds was same, the degree of interaction and binding location were found to be almost similar. The binding sites of the compounds were found to be in close proximity to the binding site of Ciprofloxacin. The variation in the bioactivity is mainly attributed to the difference in their binding site. For instance, the activity studies showed that compounds **83-85** showed comparable results with Ciprofloxacin in the case of *P. aeruginosa.* It may be due to the fact that their binding site is close to the Ciprofloxacin binding site.

The mode of action of compounds 83-85 with active site of CYP 51 of C. albicans (PDB ID: 1E9X)

The results show that the overall trend of the interaction energies of all the derivatives is in good qualitative agreement with the *in vitro* antifungal activities. Among these three compounds, compound **83** showed strong molecular interactions within active site of CYP 51 of *C. albicans* with 48.10 GOLD fitness score as

compared to other compounds **84**, **85** and reference drugs having 38.37, 40.81 and 47.68 fitness scores respectively. Compound **83** also found to have highest number of hydrophobic contacts as compared to other novel compounds and reference drug Fluconazole (**Figures 2**).

Figure 2 (a). The three molecules **83-85** docked in the binding site of Cytochrome P451 of *C. albicans*.

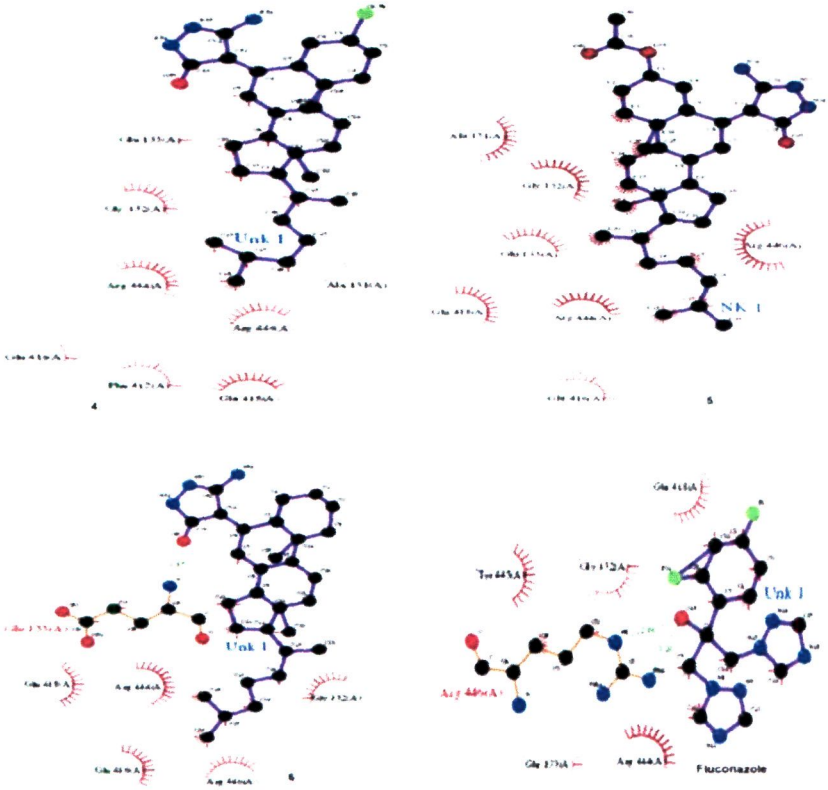


Figure 2 (b). The interaction plot of compounds **83-85** with the binding site residues of Cytochrome P451 of *C. albicans*.

The compound **83** docked within the active site with one hydrogen bond and twenty eight hydrophobic interaction with rang of 3.24-3.9Å (**Table 26**). In case of compound **83**, the pyrazolone ring forms H-bonding interactions with the residues Glu133 in the S4 subsite. The binding mode of these derivatives with the active site of Cytochrome P450 provides reasonable explanation for their antifungal activities. The antifungal activities of all the compounds are comparable to the control Fluconazole. The results shown in **Table 26** also suggest that the appropriate length of the substituent on the derivatives is also important for antifungal activities. The S4 sub site is a hydrogen bond donor and acceptor region, which interacts with the oxygen of the pyrazolone ring.

Table 26. The ligand molecules with number of molecular interactions and he scores for strength of binding with Cytochrome P451 of *C. albicans*.

Comp.	GOLD fitness score	Residues involved in Hydrogen bonding	Residues involved in Hydrophobic interaction	H-bond Range (Å)	Hydrophobic Interaction Range (Å)
83	48.10	Glu133	Ala131,Gly132,Glu133,Glu416, Glu418,Arg444, Arg446	2.85	3.24-3.90
84	38.37	NA	Ala131,Gly132,Glu133, Glu416, Arg444, Arg446	NA	3.11-3.81
85	40.81	NA	Ala131,Gly132,Glu133, Glu416, Phe417,Glu418,Arg444, Arg446	NA	3.12-3.87
Fluconazole	47.68	Arg446	Ala131,Gly132,Glu133,Glu416, Arg444, Arg446	2.96-3.90	3.43-3.82

Biological evaluation of steroidal thiazolidinone derivatives 94-96

(Refer to Chapter-4 for their synthesis and characterization)

Rule of Five and bioactivity score

The synthesized compounds **94-96** showed only one violation from rules of five for compound **94** due to a calculated Clog P value above the limit of 5 and two violations for compounds **95** and **96** due to the same reason and the molecular mass above 500. On the basis of these results we can say that the synthesized compounds reasonably follow Lipinski's "Rule of Five" (**Table 27**).[23]

Table 27. Calculated physicochemical properties of steroidal thiazolidinone derivatives **94-96**.

Comp.	Mwt	ClogP	HBD	HBA	TPSA	No. violations
94	474.80	8.08	2	3	46.332	1
95	532.8	7.41	2	4	72.405	2
96	509.24	7.90	2	3	46.332	2

The bioactivity score of the compounds **94-96** was also calculated for six criteria, GPCR ligand activity, ion channel modulation, kinase inhibition activity, protease inhibitor, enzyme inhibitor and nuclear receptor ligand activity. Normally for organic molecules if the bioactivity score is more than 0.00 then the compound is active but if it is between -0.50 to 0.00 then the compound is moderately active and if the compound has less than -0.50 then it is inactive compound. The results (**Table 28**) of compounds **94-96** showed good bioactivity score.

Table 28. Bioactivity score of steroidal thiazolidinones **94-96**.

Comp.	GPCR ligand	Ion channel	Modulator Kinase	Protease inhibitor	Nuclear receptor ligand	Enzyme inhibitor
94	-0.08	-0.12	-0.66	0.10	0.05	0.15
95	-0.04	-0.07	-0.65	0.17	0.11	0.23
96	-0.04	-0.17	-0.71	0.05	-0.01	0.13

Anticancer activity

Steroidal thiazolidinones were evaluated for cytotoxicity in a panel of selective human cancer cells using the MTT assay (**Table 29**). The panel of cancer cells encompassed HepG2 (from hepatocellular carcinoma), A549 (from lung adenocarcinoma epithelium), SW480 (from colon adenocarcinoma), HeLa (from cervical carcinoma) and HL-60 from (promyelocytic leukaemia). Doxorubicin and Cytarabine were used as cytotoxic drugs of reference. The compound **96** showed potential activity with minimum IC_{50}= 12.03±0.5 (HeLa) and 14.09±0.6 (HL-60) μmol L^{-1}

Table 29. Anticancer activity of steroidal thiazolidinones **94-96** against cancer cells.

Comp.	SW480	A549	HepG2	HeLa	HL60	PMBC
94	10.9±0.4	19.05±0.2	15.03±0.5	26.32±0.3	24±0.3	67
95	15.67±0.2	29.21±0.3	27.04±0.2	15.03±0.5	13.25±0.2	69
96	28.01±0.4	32.52±0.6	38.14±0.2	12.03±0.5	14.09±0.6	68
Doxorubicin	25.11±0.3	13.5±0.3	11.52±0.6	12.52±0.3	9.52±0.2	-
Cytarabine	15.01±0.3	16.05±0.2	13.04±0.4	14.32±0.2	10.09±0.2	-

To confirm the cytotoxicity, the synthesized compounds (**94-96**) were also tested with non-cancer cell lines **PMBC** during which none of these compounds were found toxic, all the compounds showed IC_{50}> 60 μmolL^{-1}.

Antimicrobial Activity

The steroidal thiazolidinones were screened for their *in vitro* antimicrobial activities against Gram-positive and Gram-negative bacterial strains and were found to possess activities against the microorganisms listed in **Tables 30** and **31**. All of the compounds present excellent activities against Gram-positive bacteria, exhibiting MIC values of 25-100 μg/mL. On the other hand, all the derivatives possess moderate to excellent antimicrobial activities against Gram-negative strains. Since the antibacterial activity were found almost same whatever the derivative used, suggesting that the mechanism of action of these compounds is depending on the class of bacteria considered. Among these compounds it was clear that compound **94**

showed very good antibacterial activity nearly equivalent to that of standard drug Ciprofloxacin.

Table 30. Antibacterial activity (zones of inhibition) of steroidal thiazolidinones **94-96.**

Diameter of zone of inhibition (mm).					
Comp.	**Gram positive bacteria**			**Gram negative bacteria**	
	S. Pyogenes	***MRSA****	***P. aeruginosa***	***K. pneumoniae***	***E. coli***
94	20.4±0.4	18.2±0.4	30.2±0.4	17.2±0.4	24.6±0.4
95	19.1±0.4	16.6±0.4	28.3±0.4	15.5±0.4	21.7±0.4
96	19.2±0.3	17.1±0.4	29.1±0.4	17.1±0.3	23.1±0.3
Standard	23.0±0.2	22.0±0.2	32.0±0.3	19.0±0.2	27.0±0.2
DMSO	-	-	-	-	-

Positive control (standard); Ciprofloxacin and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).

Table 31. MIC and MBC results of steroidal thiazolidinones **94-96** against bacterial strains.

Comp.	**Gram positive bacteria**				**Gram negative bacteria**					
	S. Pyogenes		***MRSA****		***P.aeruginosa***		***K. pneumonia***		***E. coli***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
94	100	>100	50	>100	50	>100	50	>100	50	>100
95	50	100	50	100	50	100	50	100	50	100
96	50	100	25	50	50	100	25	50	25	50
Standard	12.5	12.5	6.25	12.5	12.5	25	6.25	25	6.25	12.5

(Standard); Ciprofloxacin; MIC (μg/ml) = minimum inhibitory concentration, i.e the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (μg/ml) = minimum bacterial concentration, i.e., the lowest concentration of the compound for killing the bacteria completely; * Methicillin resistant *Staphylococcus aureus* (MRSA +Ve).

For assaying antifungal activity, different fungal strains such as *Candida albicans*, *Asperergillus fumigatus*, *Pencillium marneffei* and *Trichophyton mentagrophytes* were chosen. The antifungal screening data showed moderate to good antifungal activity (**Tables 32** and **33**).

Table 32. Antifungal activity of steroidal thiazolidinones **94-96**.

Diameter of zone of inhibition (mm)				
Comp.	**CA**	**AF**	**TM**	**PM**
94	25.8±0.2	22.7±0.3	16.3±0.5	14.5±0.1
95	24.8±0.1	21.7±0.3	16.6±0.2	17.5±0.3
96	21.9±0.4	16.7±0.5	14.9±0.3	11.8±0.2
Standard	30.0±0.2	27.0±0.2	24.0±0.3	20.0±0.5
DMSO	-	-	-	-

CA; *Candida albicans*, AF; *Aspergillus fumigatus*, TM; *Trichophyton mentagrophytes*, PM; *Penicillium marneffei*. Positive control (standard); Fluconazole and negative control (DMSO) measured.

Among the screened compounds, **94** and **96** were found to have good zones of inhibition. The compound **94** showed maximum inhibition against *C. albicans* and *Trichophyton mentagrophytes* strains, while compound **96** was more effective by showing maximum inhibition against *C. albicans*, *Aspergillus fumigatus* and *Penicillium marneffei* strain. The MFC of all the compounds was two or three folds higher than the corresponding MIC results.

Table 33. MIC and MFC of steroidal thiazolidinones **94-96** against fungal strains.

Comp.	**CA**		**AF**		**TM**		**PM**	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
94	25	100	50	100	50	100	50	100
95	50	100	50	100	100	>100	100	>100
96	25	50	25	100	50	100	25	100
Standard	6.25	25	12.5	12.5	6.25	25	12.5	125

(standard); Fluconazole; CA; *Candida albicans*, AF; *Aspergillus fumigatus*, TM; *Trichophyton mentagrophytes*, PM; *Penicillium marneffei*. MIC (µg/ml) = minimum inhibitory concentration, i.e the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (µg/ml) = minimum fungicidal concentration, i.e., the lowest concentration of the compound for killing the fungus completely.

In vitro antioxidant activity

The *in vitro* antioxidant activity and scavenging effects of steroidal thiazolidinones were evaluated by using different reactive species assay containing DPPH radical scavenging activity. The free radical scavenging activity of all the synthesized compounds were evaluated through their ability to quench the DPPH• using ascorbic acid as reference. The potencies for the antioxidant activity of the synthesized compounds to the reference drug are shown in **Table 34.**

Table 34. The antioxidant activity data of steroidal thiazolidinones **94-96**[a].

	% inhibition			
Comp.	**25 µg/mL**	**50 µg/mL**	**75 µg/mL**	**100 µg/mL**
94	27.1±0.4	22.5±0.3	25.6±0.3	21.5±0.4
95	14.8±0.2	11.6±0.5	12.6±0.5	10.9±0.9
96	18.2±0.7	18.2±0.4	21.6±0.1	15.5±0.3
Standard	36.0±0.3	37.0±0.2	44.0±0.3	50.0±0.5
Control[b]	-	-	-	-

[a]Values represent the mean ± standard error mean (SEM) of three experiments.
[b]No inhibition, standard: ascorbic acid.

Among these compounds, compound **94** exhibited a slightly better antioxidant activity than **95** and **96.**

Molecular Docking

Molecular Docking of compounds 94-96 with S12 protein (PDB ID: 1FJG)

The potent activity of steroidal thiazolidinones as new antimicrobial agents, prompted us to study the docking of these derivatives inside the active site of S12 protein. X-ray study of S12 with divergent inhibitors showed that the binding pocket of the protein included the following residues Arg 89, Lys 46, Lys 47 and Lys 91 as shown in **Figures 3.** The top score was selected for each compound and the results were shown in **Table 35** and also compared with Ciprofloxacin, which was redocked with the target protein using the same protocol. It was evident from the **Figures 3** that for each compound, the binding site was found to be the same. It was interesting to observe that even though the core structure of all the compounds was same, the degree of interaction and binding location were found to be different for these compounds. The binding sites of the compounds were found to be in close proximity

to the binding site of Ciprofloxacin. The variation in the bioactivity is mainly attributed to the difference in their binding pattern.

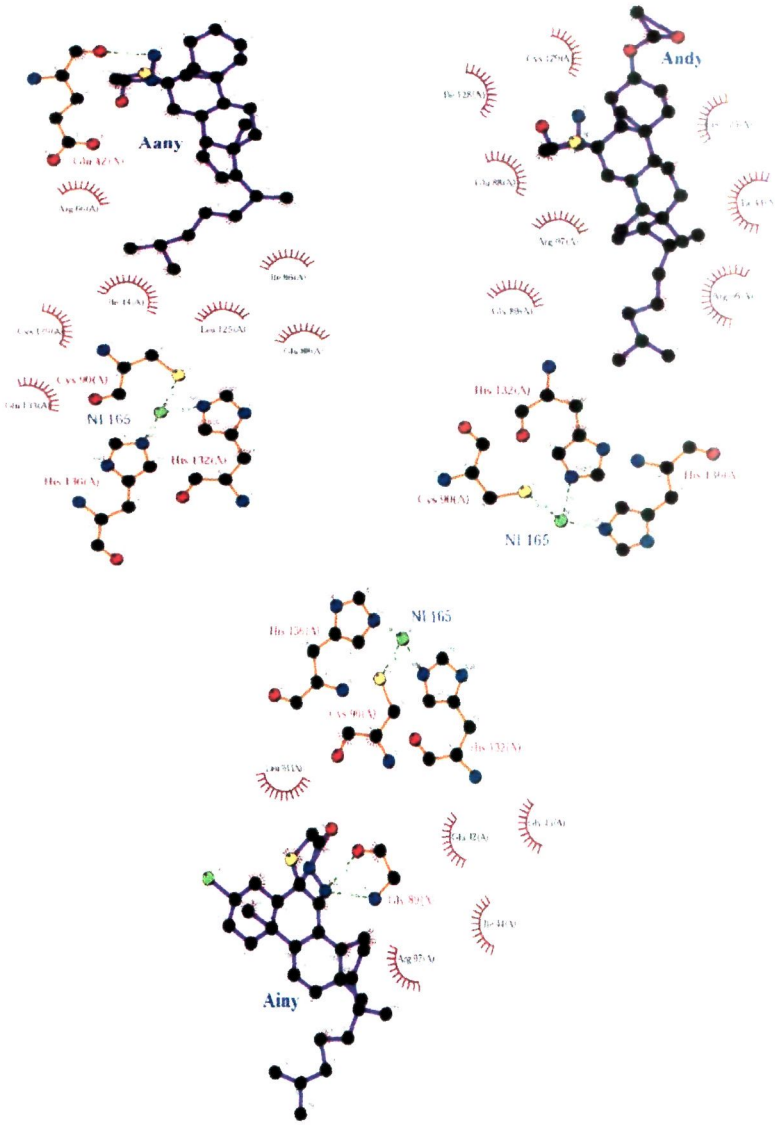


Figure 3 (a). The three molecules (**94-96**) docked in the binding site of S12 protein.

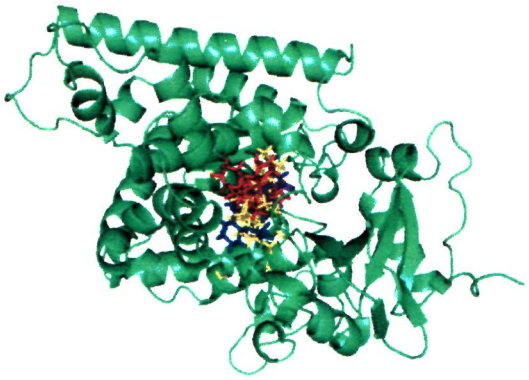


Figure 3 (b). The interaction plot of all the three molecules (**94-96**) with the binding site residues of S12 protein

Table 35. The ligand molecules with number of molecular interactions and the scores for strength of binding with S12 protein.

Comp.	GOLD fitness score	Hydrogen Bond Interaction	Lipophilic Interaction	Non Bonded Interaction	XScore (kcal/mol)
94	31.4	3, Cys 90, His 132, His 136	8, Ile 44, Arg 66, Glu 88, Gly 89, Arg 97, Leu 125, Ile 128, Cys 129	24	-6.83
95	14.88	4, Glu 42, Cys 90, His 132, His 136	7, Ile 44, Arg 66, Ile 86, Glu 88, Leu 125, Cys 129, Glu 133	25	-6.81
96	20.83	3, Cys **90**, His 132, **His** 136	5, Glu 42, Gly 43, Ile 44, Leu 91, Arg 97	18	-6.77
Ciprofloxacin	50.31	1, His 101	4; Lys 97, Ala 256, Cys 394, Val 395	22	-8.06

Molecular docking of compounds 94-96 with active site of CYP 51 of C. albicans (PDB ID: 1E9X)

The binding site of Fluconazole (ligand present in the template) is used as the active site in the Cytochrome P450-14DM model (**Figures 4)**. The results show that the overall trend of the interaction energies of all the derivatives is in good qualitative agreement with the *in vitro* antifungal activities. Among the synthesized compounds, compound **94** had maximum negative Glide score as well as maximum binding energy with the active site of Cytochrome P450 14DM of *C. albicans* (**Table 36**). These compounds also showed better bioactivity results as compared to standard Fluconazole.

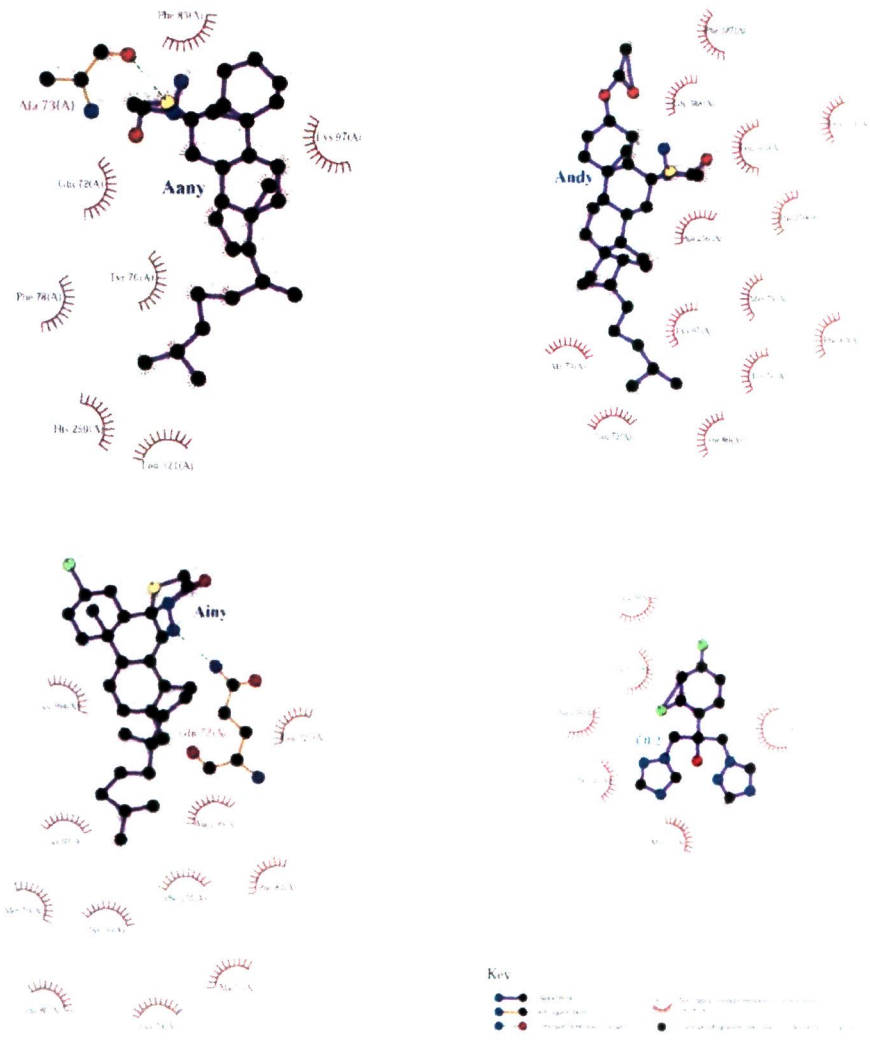


Figure 4 (a). All the molecules (**94-96**) docked in the binding site of CYP 51 protein.

Figure 5 (b). The interaction plot of all the molecules (**94-96**) with the binding site CYP 51protein.

Table 36. The ligand molecules (**94-96**) with number of molecular interactions and the scores for strength of binding with CYP51 protein.

Comp.	GOLD fitness score	Hydrogen Bond Interaction	Lipophilic Interaction	Non-Bonded Interaction	XScore (kcal/mol)
94	48.2	1, Gln 72	11, Ala 73, Lys 74, Tyr 76, Met 79, Thr 80, Phe 83, Lys 97, Phe 255, Ala 256, Leu 321, Cys 394	22	-5.89
95	41.16	1, Ala 73	7, Gln 72, Tyr 76, Phe 78, Phe 83, Lys 97, His 259, Leu 321	29	-5.97
96	43.23	NIL	13, Gln 72, Ala 73, Tyr 76, Met 79, Thr 80, Phe 83, Lys 97, Ala 256, His 259, Leu 321, Leu 324, Phe 387, Gly 388	32	-6.17
Fluconazole	36.71	NIL	6; Tyr 76, Met 79, Phe 255, Ala 256, His 259, Leu 321	24	-7.35

Experimental

Rule of Five and bioactivity score

The physicochemical parameters including octanol partition coefficients (CLogP), Mwt, HBD, HBA and TPSA as well as bioactivity score were calculated using molinspiration server (http://www.molinspira tion.com/cgi-bin/properties) and ChemAxon (chemicalize.org).[27]

Anticancer activity

Cell lines and culture conditions

Human cancer cell lines SW480 (human colon adenocarcinoma cells), HeLa (human cervical cancer cells), A549 (human lung carcinoma cells), HepG2 (human hepatic carcinoma cells), and HL-60 (human leukaemia) were taken for the study. Doxorubicin and Cytarabine were used as cytotoxic drugs of reference. SW480, A549, HL60 and HepG2 cells were grown in RPMI 1640 supplemented with 10 % fetal bovine serum (FBS), 10U penicillin and 100 µg/mL streptomycin at 37 °C with 5 % CO_2 in a humidified atmosphere. HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplanted with FCS and antibiotics as described above for RPMI 1640. Fresh medium was given every second day. Cells were passaged at preconfluent densities using a solution containing 0.05 % trypsin and 0.5 mM EDTA.

Blood peripheral mononuclear cell isolation (PBMC)

Fresh blood (20–15 mL) was kindly provided by the Blood Bank Jawahar Lal Nehru Medical College, AMU Aligarh. The blood sample was diluted with the same volume of phosphate buffered saline (PBS) (1.5 KH_2PO_4, 6.5 Na_2HPO_4, 137 NaCl, and 2.7 mM KCl; pH 7.4). Then the diluted blood sample was carefully layered on Ficoll-Histopaque. The mixture was centrifuged at 400g for 30 min at 20-22 °C. The undisturbed lymphocyte layer was carefully transferred out. The lymphocyte was washed and pelleted down with 3 v of PBS for twice and resuspended RPMI-1640 media with antibiotic and antimycotic solution 10 %, v/v FCS. Cell counting was performed to determine the PBMC cell number with equal volume of trypan blue.

Cell viability assay (MTT)

The anticancer activity *in vitro* was measured using the MTT assay. Exponentially growing cells were harvested and plated in 96-well plates at a concentration of 1×10^4 cells/well. After 24 h incubation at 37 °C under a humidified 5 % CO_2 to allow cell attachment, the cells in the wells were respectively treated with target compounds at various concentrations for 48 h. The concentration of DMSO was always kept below 1.25 %, which was found to be non-toxic to the cells. A solution of

3-(4,5-dimethylthizao1-2-yl)-2,5-diphenyltetrazolium bromide (MTT), was prepared at 5 mg/mL in phosphate buffered saline (PBS; 1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl; pH 7.4). 20 µL of this solution were added to each well. [28] After incubation for 4 h at 37 °C in a humidified incubator with 5% CO_2, the medium/MTT mixtures were removed, and the formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in 100 µL of DMSO per well. The absorbance of the wells was read with a microplate reader (Bio-Rad Instruments) at 570 nm. Effects of the drug cell viability were calculated using cell treated with DMSO as control.

Data analysis

Cell survival was calculated using the formula: Survival (%) = [(absorbance of treated cells-absorbance of culture medium) / (absorbance of untreated cells - absorbance of culture medium)] ×100.[28] The experiment was done in triplicate and the inhibitory concentration (IC) values were calculated from a dose response curve. IC_{50} is the concentration in 'µM' required for 50 % inhibition of cell growth as compared to that of untreated control. IC_{50} values were determined from the linear portion of the curve by calculating the concentration of agent that reduced absorbance in treated cells compared to control cells by 50 %. Evaluation is based on mean values from three independent experiments, each comprising at least six microcultures per concentration level.

Antimicrobial Activity

The synthesized compounds were screened for their *in vitro* antibacterial activities against the culture of *Streptococcus pyogenes* (ATCC-29213), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Escherichia coli* (ATCC-25922) and *Kliebsiella pneumoniae* (Clinical isolate) by disk diffusion method.[23] The minimum inhibitory concentration (MIC) of all the compounds was determined. Ciprofloxacin (30 mg) was used as positive control, whereas DMSO poured disk was used as negative control and then minimum inhibitory concentration (MIC) was evaluated by the macro-dilution test using standard inoculums of 1-2 $\times 10^7$ c.f.u. mL^{-1} (0.5 McFarland standards). Serial dilutions of the test compounds, previously dissolved in dimethyl sulfoxide (DMSO) were prepared to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 $mgmL^{-1}$. To each tube was added 100 mL of 24 h old inoculums. The MIC, defined as the lowest concentration of the test compound which inhibits the visible growth after 18 h and it

was determined visually after incubation for 18 h, at 37 °C. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 37 °C. The tests use DMSO and Ciprofloxacin as negative and positive controls, respectively. The *in vitro* antifungal activities of synthesized compounds were carried out using *Candida albicans, Aspergillus fumigatus, Trichophyton mentagrophytes* and *Pencillium marneffei* (recultured) in DMSO by agar diffusion method.[23] The minimum inhibitory concentration (MIC) was determined by broth dilution technique as in antibacterial activity. The Inhibition zones of compounds were compared with Fluconazole used as standard drug. The nutrient broth which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately 1.6-6 $\times 10^4$ c.f.u. mL^{-1}. The cultures were incubated for 48 h at 37 °C and the growth was monitored.

Antioxidant activity

The synthesized compounds were tested for their antioxidant property by 1,1-diphenylpicrylhydrazyl (DPPH) method. Drug stock solution (1 mg/mL) was diluted to final concentration of 2, 4, 6, 8, 10 and 12 mg/mL in methanol. Methanolic DPPH solution (1 mL, 0.3 mmol) was added to 3.0 mL of drug solution of different concentrations. The tube was kept at an ambient temperature for 30 min and the absorbance was measured at 517 nm. The scavenging activity was calculated by the following formula:

$[\%\text{inhibition} = [(A_{Control} - A_{Sample}/A_{Control} \times 100)]$

where $A_{Control}$ is the absorbance of the L-ascorbic acid (Standard) and A_{Sample} is the absorbance of different compounds. The methanolic DPPH solution (1 mL, 0.3 mM) was used as control.[29]

Docking study

Protein and ligand preparation

The three dimensional structures of the targets were downloaded from protein databank. Hydrogen atom and MMFF partial charge were added to enzyme. Potential steric clashes and added hydrogen atoms were relaxed by using the minimization procedure. The minimization was performed by using a CHARMm force field with Dependent Dielectric implicit solvent model and conjugates gradient method. This process was carried out until the average absolute derivative of co-ordinates with respect to energy fell below the 0.1 kcal $Å^{-1}$. The two dimensional structures of ligands were prepared by using the ChemDraw Ultra 11.0 software integrated with

Cambridgesoft Software (Cambridgesoft Corporation).[30] Further refinement of compounds was performed by using energy minimization protocol with cvff force field.

Molecular Docking

GOLD (Genetic Optimization for Ligand Docking) 5.0 was used for docking of the compounds dataset against selected targets in present study.[31] Docking annealing parameters for van der Walls and hydrogen bonding were set to 5.0 and 2.5 respectively. The parameters used for genetic algorithm were population size 100, selection pressure 1.2, number of operations 1,00,000, number of islands 5, niche size 2, migrate 10, mutate 100 and cross-over 100. Interaction analyses were performed by using Ligplot figures of the complexes and prepared by using discovery studio visualizer.[32]

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LIST OF PUBLICATIONS

1. **3β-Acetoxy-6-nitrocholest-5-ene: Crystal structure, thermal, optical and dielectrical behavior**
   Shamsuzzaman, **Ashraf Mashrai**, Hena Khanam, Yahia Nasser Mabkhot, Wolfgang Frey.
   J. Mol. Str., 1063 (**2014**) 219–225.

2. **Synthesis, evaluation and docking studies on steroidal pyrazolones as anticancer and antimicrobial agents**
   Shamsuzzaman, **Ashraf Mashrai**, Anis Ahmad, Ayaz M. Dar, Hena Khanam, Mohd Danishuddin, Asad U. Khan.
   Med. Chem. Res., 23 (**2014**) 348-362

3. **Biological synthesis of ZnO nanoparticles using *C. albicans* and studying their catalytic performance in the synthesis of steroidal pyrazolines**
   Shamsuzzaman, **Ashraf Mashrai**, Hena Khanam, Rezq N. Aljawfi.
   Arabian J. Chem., (**2013**) (in press);
   http://dx.doi.org/10.1016/j.arabjc.2013.05.004

4. **Green synthesis and biological evaluation of steroidal 2H-pyrans as anticancer and antioxidant agents.**
   Shamsuzzaman, **Ashraf Mashrai**, Hena Khanam, Mohd Asif, Abad Ali, Asif Sherwani, Mohammad Owais.
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5. **Synthesis, growth, spectral, thermal and crystallographic studies of 5α,6α-epoxycholestane single crystals**
   Shamsuzzaman, Hena Khanam, **Ashraf Mashrai**, Musheer Ahmad, Yahia N. Mabkhot,Wolfgang Frey, Nazish Siddiqui.
   J. Cryst. Growth, 384 (**2013**) 135-143

6. **Synthesis and anti-tumor evaluation of B-ring substituted steroidal pyrazoline derivatives**
   Shamsuzzaman, Hena Khanam, **Ashraf Mashrai**, Asif Sherwani, Mohammad Owais, Nazish Siddiqui.
   Steroids, 78 (**2013**) 1263-1272

7. **Construction of novel steroidal isoxazolidinone derivatives under Vilsmeier-Haack conditions**
   Shamsuzzaman, Hena Khanam, **Ashraf Mashrai**, Nazish Siddiqui.
   Tetrahedron Lett., 54 (**2013**) 874-877

8. **6-Hydroxyimino-5α-cholestane**
   Shamsuzzaman, Hena Khanam, **Ashraf Mashrai**, Yahia N. Mabkhot, Ahmad Husain.
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3β-Acetoxy-6-nitrocholest-5-ene: Crystal structure, thermal, optical and dielectrical behavior

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HIGHLIGHTS

- 3β-Acetoxy-6-nitrocholest-5-ene has been synthesized from 3β-acetoxycholest-5-ene.
- Structural assignment has been performed on the basis of FT-IR, FT-Raman, ^{1}H NMR, ^{13}C NMR, and X-ray crystallography.
- Thermal, optical, microstructural and dielectrical properties have also been explored.
- Thermal studies (TG–DTA–DSC) showed thermal stability and good crystalinity of the compound.

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ABSTRACT

3β-Acetoxy-6-nitrocholest-5-ene (**2**) has been synthesized from 3β-acetoxycholest-5-ene (**1**). We provided an analysis of the compound by means of FT-IR, FT-Raman, NMR and X-ray crystallography. In addition microstructural, thermal, optical and dielectrical properties were also investigated. The compound **2** crystallizes in the monoclinic space group $P2_1$ with cell dimensions, $a = 15.7729(13)$ Å, $b = 9.8933(8)$ Å, $c = 17.8070(14)$ Å, $\alpha = 90.00$, $\beta = 96.176(4)$, $\gamma = 90.00$. The powder X-ray diffraction (PXRD) of the compound was recorded to ascertain phase homogeneity. The SEM micrograph showed the presence of brick shaped, elongated nitrocholestane particles with 177.12 × 25.53 × 5.69 μm dimensions. Thermogravimetric analysis showed stability of the compound up to 200 °C. The dielectrical studies showed that with increase in frequency, the dielectric constant decreases and becomes almost constant at high frequencies.

1. Introduction

Steroids, a widespread class of natural organic compounds occurring in animals, plants and fungi, have shown great therapeutic value for a broad array of pathologies. They comprise a wide repertoire of structurally related natural compounds with important functions *in vivo*, such as physiological regulators, hormones, and provitamins. Steroids are representative of a rich structural molecular diversity and ability to interact with various biological targets and pathways. For the last seventy years the chemistry of steroids has provided one of the most interesting and thoroughly explored areas for organic chemists. The synthetic modification of naturally occurring steroids with the hope of improving pharmacological essentials has resulted in the preparation and discovery of a number of diverse pharmacologically active, potent, highly specific commercially important therapeutic agents [1–3]. Moreover steroids play an important biological role and have occupied a prominent position in medicinal chemistry field. Steroids have always attracted considerable interest because of their biological signaling molecules. Many representatives of this group are widely used in medicine as essentials of anti-inflammatory, diuretic, anabolic, contraceptive, antiandrogenic, progestational, anticancer and antimicrobial agents [4,5]. The steroidal drugs are widely used in traditional medicines, such as antibacterium, hormone kind medication, etc. Some steroid compounds are known to exert hormone receptor-independent antiproliferative activity via the inhibition of angiogenesis, tubulin polymerization and the upregulation of apoptotic pathways [6–8]. Cholesterol (Chol), a well known steroid, is ubiquitous in all living systems. Cholesterol is located in all membrane compartments at levels as high as 50-mole percent, which renders it the most prominent lipid in eukaryotic cells [9]. Cholesterol, which is synthesized de novo and obtained from the diet, largely affects the biophysical properties of cellular membranes and functions in a variety of synthetic pathways, including

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, evaluation and docking studies on steroidal pyrazolones as anticancer and antimicrobial agents

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Abstract A series of new steroidal pyrazolones have been synthesized, characterised and evaluated for their in vitro anticancer activity. They were tested against five cancer (SW480, HepG2, A549, HeLa and HL-60) cell lines. The synthesized compounds showed high selectivity and compound **4** showed the strongest inhibitory activity against human SW480 (IC_{50} = 11.67 μmol L^{-1}). In addition, the synthesized compounds were tested for their antimicrobial activity by disc diffusion assay and MIC by broth micro dilution method against Gram-positive, Gram-negative strains of bacteria as well as fungus strains and we found a correlation between the observed and predicted antimicrobial activities. Docking studies were performed to investigate the hypothetical binding mode of the target compounds. This study provided a new molecular scaffold for the further development of anticancer as well as antimicrobial agents.

Keywords Steroid · Pyrazolone · Anticancer · Antimicrobial · Docking

Introduction

Steroids attract much attention in cell biology and pathophysiology because of the wide range of biological phenomena in which they are involved (Vejux and Lizard, 2009). The involvement of steroids in anticancer promotion and suppression has been known for a long time. This involvement goes far beyond the steroidal sex hormones (Salvador *et al.*, 2013). Many anticancer steroids are enzyme inhibitors, such as aromatase and sulfatase inhibitors for breast cancer, 5 α-reductase inhibitors for the treatment of benign prostatic hyperplasia and CYP 17 inhibitors for advanced prostate cancer therapy (Handratta *et al.*, 2005). A variety of steroids with unusual and interesting structures have been synthesized and evaluated for their antitumor activity (Krstic'a *et al.*, 2007; Poza *et al.*, 2007; Bansal and Guleria, 2008; Koutsourea *et al.*, 2008; Thibeault *et al.*, 2008). Among these steroids, nitrogen containing steroid derivatives have been shown to be more potent and have been used clinically for the treatment of cancer (Guarna *et al.*, 1999; Ling *et al.*, 1997). Among all the numerous antibiotics developed to date, few compounds possessing a steroid nucleus have been studied and the results clearly showed that these compounds exhibited a good antibacterial activity against several human pathogenic bacteria (Jayasinghe *et al.*, 1998; Atta *et al.*, 1998). The potent mechanism of action of these compounds described by the interactions of amine groups with the negative phosphate groups of LPS displacing divalent cations such as Ca^{2+} and Mg^{2+} (Nikaido, 1996; Vaara, 1993). Pyrazolones are important structural cores in many drug substances of medicinal fields. Heterocyclic nucleus containing pyrazolones are useful antipyretic and analgesic drugs (Himly *et al.*, 2003), whilst edaravone (MCI-186) has been used for treating the brain (Kawai *et al.*, 1997) and myocardial ischemia (Wu *et al.*, 2002). In addition, pyrazolones possess kinase inhibitory properties, particularly of enzymes which catalyze the phosphorylation of serine and threonine in proteins and also used for

Electronic supplementary material The online version of this article (doi:10.1007/s00044-013-0636-y) contains supplementary material, which is available to authorized users.

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ORIGINAL ARTICLE

Biological synthesis of ZnO nanoparticles using *C. albicans* and studying their catalytic performance in the synthesis of steroidal pyrazolines

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Steroid;
Pyrazoline

Abstract In this study, we describe a green and simple procedure for biosynthesis of ZnO nanoparticles using *Candida albicans* as eco-friendly reducing and capping agent. The synthesized ZnO nanoparticles were characterized by UV–vis spectroscopy, powder X-ray diffraction, scanning electron microscopy (SEM), transmission electron microscopy (TEM), photoluminescence (PL), thermo gravimetric analysis (TGA) and differential thermal analysis (DTA). The prepared nano-particles were used as catalyst for the fast and efficient synthesis of steroidal pyrazolines (**4–9**) from α, β-unsaturated steroidal ketones (**1–3**). The target molecules were obtained in good to excellent yields applying the current method.

1. Introduction

Green chemistry can be recognized as pioneering research, which widely reports intrinsic atom economy, energy savings, waste reduction, easy work ups and the avoidance of hazardous chemicals (Kumar et al., 2012). The development of a simple, eco-friendly reaction protocol for the synthesis of highly functionalized compound libraries of medicinal motifs is an attractive area of research in both academia and the pharmaceutical industry (Domling, 2006). Nano-chemistry is an up growing research area due to its unique properties (Xia et al., 2003). The usage of nanomaterials as catalyst has gained a significant role in organic synthesis due to simple work-up procedure, environmentally benign nature, reusability, low cost, and ease of isolation. Recently, nano-crystalline inorganic oxides exhibited enormous opportunities and attracted the interest of several research groups because of their different topical characteristics and wide range of particle sizes (Hadjipanayis and Siegel, 1994). Of late, catalysis by nanomaterials has become an area of interest, as these materials exhibit better catalytic activity compared to their bulk sized counterparts (Beydoun et al., 1999; Zhang et al., 2007). Zinc oxide as a non-toxic, inexpensive, and non-hygroscopic polar inorganic crystalline material is very economical, safe, and easily available Lewis acid catalyst, which has gained much interest in various organic transformations,

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ORIGINAL ARTICLE

Green synthesis and biological evaluation of steroidal 2*H*-pyrans as anticancer and antioxidant agents

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Abstract In this study, we describe a green and simple procedure for the synthesis of steroidal 2*H*-pyrans **4–6** using chitosan as an eco-friendly heterogeneous catalyst. The synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS and analytical data. These compounds were tested *in vitro* against two cancer cell lines [HeLa (cervical) and Jurkat (leukemia)] and one normal cell line (PBMC). The compounds exhibited moderate to good activity against the two human cancer cell lines and were less toxic against the non-cancer cell line. In addition, the synthesized compounds were tested for their *in vitro* antioxidant activity by 1,1-diphenylpicrylhydrazyl method in which compounds **4** and **6** exhibited good antioxidant activity. This study provided a new molecular scaffold for the further development of anticancer as well as antioxidant agents.

1. Introduction

Green chemistry can be recognized as a pioneering research, which widely reports intrinsic atom economy, energy savings, waste reduction, easy work ups and the avoidance of hazardous chemicals (Kumar et al., 2012). The progress of a simple and eco-friendly reaction protocol for the synthesis of highly functionalized compound libraries of medicinal motifs is an attractive area of investigation in both academia and the pharmaceutical industry (Domling, 2006). Chitosan is readily prepared *via* aqueous alkali promoted hydrolysis of chitin. Being hydrophilic and possessing basic moieties (Bader and Birkholz, 1996), chitosan has been utilized as a heterogeneous eco-friendly basic catalyst for reactions carried out in protic medium (Gomha and Riyadh, 2009). Steroids are an important class of natural products which have high capability to penetrate cells and bind to nucleus and membrane receptors. They include great variations in structure and play a very important role in life (Festi et al., 2007; Ifere et al., 2009). The investigation of modified steroid derivatives condensed with different heterocyclic rings has drawn great attention (El-Far et al., 2009). Pyrans and their derivatives constitute an important class of organic compounds due to their attractive pharmacological and biological properties (Green et al., 1995; Nemouchi et al., 2012). They are widely used as anticoagulant, anticancer, diuretic, spasmolitic and antianaphylactin

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Synthesis, growth, spectral, thermal and crystallographic studies of 5α,6α-epoxycholestane single crystals

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ABSTRACT

α-Selective epoxidation of 3β-chlorocholest-5-ene and 3β-acetoxycholest-5-ene has been performed with m-CPBA to synthesize 3β-chloro-5α,6α-epoxycholestane (**1**) and 3β-acetoxy-5α,6α-epoxycholestane (**2**). We provided an analysis of these compounds by means of FT-IR, FT-Raman, ^{1}H NMR, ^{13}C NMR, 2D cosy, NOESY, UV–visible and X-ray crystallography. The compound **1** crystallizes in the orthorhombic space group $P2_12_12_1$ while compound **2** crystallizes in the monoclinic space group $P2_1$. We compared the conformations of both compounds in solid state and in solution by calculation of dihedral angles and coupling constant values. The powder X-ray diffraction (PXRD) of the compound was recorded to ascertain the purity of the grown crystals. Thermogravimetric analysis showed stability of the compounds up to 250 °C. Moreover, the ICH rule has been applied to test the stability of two crystals, which showed significant stability.

1. Introduction

Cholesterol is a tetracyclic lipid of biological importance since its discovery by François Poulletier de la Salle in 1758. Since the last century cholesterol is known to be subject to oxidation leading to the formation of mono- or poly-oxygenation products called oxysterols. Oxysterols attract much attention in cell biology and pathophysiology because of the wide range of biological phenomena in which they are involved. Oxysterols can be endogenously produced from cholesterol (Chol[4]) by enzymatic and nonenzymatic oxidative processes or absorbed from diet sources [1]. They participate in the biosynthesis of bile acids and steroid hormones, acting also as signaling lipids that regulate cholesterol biosynthesis, cellular cholesterol efflux, lipoprotein uptake, intracellular cholesterol trafficking [2] and have been related to neurodegenerative diseases, such as Alzheimer, Parkinson, and Multiple sclerosis [1]. Most common oxysterols are known to interfere with the cell membrane structure and cellular receptors [3] to inhibit cholesterol [2,4] and DNA biosynthesis [5], and to induce cell death in different cell lines by apoptosis or necrosis [1].

Among oxysterols, 5,6-epoxycholestanes have stimulated the interest of researchers some years after the photo-oxidation products of cholesterol were suspected to be involved in photocarcinogenesis [6]. Because of the presence of an oxirane group, it was supposed that 5,6-epoxycholestane could be electrophilic and behave like alkylating agents with direct carcinogenic properties. Recent data from literature ruled out that 5,6-epoxycholestanes could be direct alkylating substances [7] and provides evidence that 5,6-epoxycholestanes may be involved in physiological processes that result in metabolites with tumor promoter properties as well as to the production of steroidal alkaloids which are anti-oncogenic. Ring B oxysterols were reported to stimulate cholesterol ester formation in cultured fibroblasts [8] and 5α,6α-epoxycholestane was shown to be the most potent allosteric activator for ACAT-1 (acyl-coA: cholesterol acyl transferase) whereas 5β,6β-epoxycholestane was found to be inefficient [9]. It was found that 5α,6α-epoxycholestane has tighter interactions with phospholipids than 5β,6β-epoxycholestane and would be considered a better raft-stabilizing sterol [10]. 5α,6α-Epoxycholestane was reported to inhibit Topoisomerase II [11]. Furthermore, ring

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Synthesis and anti-tumor evaluation of B-ring substituted steroidal pyrazoline derivatives

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ABSTRACT

The synthesis and anti-tumor activity screening of new steroidal derivatives (**4–18**) containing pharmacologically attractive pyrazoline moieties are performed. During *in vitro* anticancer evaluation, the newly synthesized compounds displayed moderate to good cytotoxicity on cervical and leukemia cancer cell lines. In addition these compounds were found to be nontoxic to normal cell (PBMCs) ($IC_{50} > 50$ μM). The structure–activity relationship is also discussed. The most effective anticancer compound **9** was found to be active with IC_{50} value of 10.6 μM. It demonstrated significant antiproliferative influence on Jurkat cell lines. The morphological changes and growth characteristics of HeLa cells treated with compound **4** were analyzed by means of SEM.

1. Introduction

Cancer is becoming the biggest health hazard for the world. Despite recent advances in early diagnosis, prevention and therapy, cancer still affects millions of people worldwide and is one of the leading causes of death. In 2008, 7.6 million people died from cancer according to the World Health Organization (WHO) and without immediate action, the global number of deaths from cancer will increase by nearly 80% by 2030 [1]. Although cancer chemotherapy has established a new era of molecularly targeted therapeutics, the efficacy of the existing drugs for the treatment of various cancers is rather limited [2], and there is a need to develop new therapeutic agents to overcome the limitations with the current therapy. That's why there is an intense effort in cancer research to design new, potent, selective and less toxic anticancer agents that are capable of rapid destruction of tumor vasculature leading to tumor necrosis and anti-tumor efficacy [3,4]. *In vitro* studies, using a variety of human cancer cell lines, have been employed to evaluate the effectiveness of new medicinal compounds against these cancers.

Steroids are an important class of natural products which have high ability to penetrate cells and bind to nucleus and membrane receptors. They include great variations in structure and play a very important role in life [5,6]. Structurally diverse cytotoxic and cytostatic steroids are very relevant as lead compounds and molecular probes for anticancer drug discovery and cancer molecular mechanisms elucidation. The chemistry of steroids has motivated extensive investigation through decades and a comprehensive review on the syntheses of novel bioactive steroids has been recently published [7]. The investigation of modified steroid derivatives condensed with various heterocyclic rings has drawn great attention [8]. A variety of steroids with unusual and interesting structures have been synthesized and evaluated for their antitumor [9,10], antimicrobial [11] and anti-parasitic activities [12]. Hybrid anti-cancer agents, which combine two active compounds in one, such as steroidal alkylators, contain steroidal moiety as biological vectors for anti-tumor agents in order to diminish toxicity and to enhance specificity, were recently demonstrated [13]. Such types of agents attain duplicate effects on cancer cells. These merged molecules may act on multiple therapeutic targets and offer the possibility of circumventing drug resistance. In addition, the hybrids may also minimize unwanted side effects and allow for synergic action [14].

Pyrazolines present an interesting group of compounds, which has been known to possess wide spread pharmacological properties [15]. Recently, different authors worldwide have reported antitumor, antiproliferative or anticancer potential of pyrazoline derivatives [16–18]. These derivatives are also well known for their

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Construction of novel steroidal isoxazolidinone derivatives under Vilsmeier–Haack conditions

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ABSTRACT

A novel expeditious and convenient synthesis of 5α-cholestano-[5,6-*c*]-isoxazolidin-5′-ones based on the reaction of 5α-6-hydroxyiminocholestanes with Vilsmeier–Haack reagent (DMF/$POCl_3$) is described. The systems presented here, are novel scaffolds and have not been described before. Structural assignment of newly synthesized compounds was performed by IR, ^{1}H NMR, ^{13}C NMR, 2D ^{1}H–^{1}H COSY, MS and analytical data.

Isoxazolidinones are well-established building blocks in synthetic organic chemistry. One of the reasons the isoxazolidinones, particularly 5-isoxazolidinones, are of considerable interest to organic chemists is that they are good precursors to unnatural β-amino acids: these are, indeed, unmasked forms of 5-isoxazolidinones. These structures exhibit a wide range of biological activities.[1,2] These type of compounds are an important class of heterocyclic structures, that can be applied in drug and pharmaceutical fields. These compounds have attracted scientific interest because of their potential cytotoxic, pro-apoptotic and antimicrobial capabilities.[3] Furthermore, they can be used for the preparation of nucleoside analogues.[4] Nucleoside analogues have emerged in recent years as highly promising candidates for the development of new efficient drugs against cancer and viral infections, particularly that of the HIV.[5] Moreover, Parnafungins, natural products containing an isoxazolidinone ring, have been isolated from *Fusarium larvarum* and have been shown to be potent inhibitors of the fungal polyadenosine polymerase.[6] Because of the importance of these scaffolds in synthetic organic chemistry and their usefulness as pharmacological molecules, much attention has been focused on their synthesis.

Synthetic routes to them are numerous, including the enantioselective conjugate addition of hydroxylamines to pyrazolidinone acrylamides,[7] propenoates,[8] crotonic acid esters[9] and α,β-unsaturated-δ-lactones.[10] The 1,3-dipolar cycloaddition of nitrones with ynolates to give isoxazolidinones has been developed quite recently.[11] Significant effort continues to be directed into the development of efficient methodologies to new isoxazolidinone-based structures.

The Vilsmeier–Haack reagent (halomethyleniminium salt) formed from the interaction of dialkyl formamide such as DMF with $POCl_3$ has attracted the attention of synthetic organic chemists since its discovery in 1927.[12] It is one of the most commonly used reagents for the introduction of an aldehydic (CHO) group into electron rich aromatic systems.[13] However, the scope of the Vilsmeier reagent is not confined to the aromatic formylation reaction alone. A wide variety of alkene[14] derivatives, carbonyl[15] compounds, activated methyl and methylene[16] groups exhibit reactivity towards the Vilsmeier reagent. In addition to the carbon nucleophiles, some oxygen[17] and nitrogen[18] nucleophiles are also reactive towards Vilsmeier reagent. Numerous transformations of the iminium salts into products other than aldehydes have been achieved[19,20] and these transformations enhance the scope and versatility of the Vilsmeier–Haack reaction. Following our interest on the synthesis of new steroidal derivatives[21] we herein report a prompt and novel strategy for the synthesis of 5α-cholestano-[5,6-*c*]-isoxazolidin-5′-ones (**7–9**) based on the reaction of 5α-6-hydroxyiminocholestanes (**4–6**) with Vilsmeier reagent. Interestingly, the reaction proceeded smoothly and the desired steroidal 5′-isoxazolidinone derivatives (**7–9**) were obtained in good yield (80–87%). With the best of our knowledge there are no reports, however, describing the synthesis of steroidal 5′-isoxazolidinones via Vilsmeier–Haack reaction.

The 5α-6-hydroxyiminocholestanes[22] (**4–6**) employed for the present investigation, were conveniently obtained from the corre-

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6-Hydroxyimino-5α-cholestane

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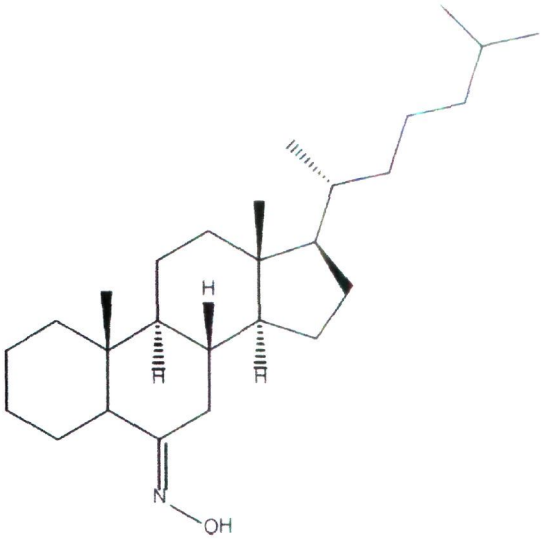
Key indicators: single-crystal X-ray study; T = 100 K; mean σ(C–C) = 0.003 Å; disorder in main residue; R factor = 0.049; wR factor = 0.129; data-to-parameter ratio = 13.7.

The title compound, $C_{27}H_{47}NO$, is a steroid derivative composed of a saturated carbon fused-ring framework with an alkyl side chain. Ring bond lengths have normal values with an average of 1.533 (2) Å, while the cholestane side chain shows an average bond length of 1.533 (2) Å. The three cyclohexane rings adopt chair conformations or close to chair conformations while the cyclopentane ring is twisted. The cholesterol side-chain is fully extended with a *gauche–trans* conformation of the terminal methyl groups. There are eight chiral centres in the molecule; the absolute configuration of these sites was determined from the structure presented. There are two molecules in the asymmetric unit; in one, the alkyl chain is disordered over two sets of sites [occupancy ratios of 0.50:0.50 and 0.67:0.33].

Related literature

For background on steroidal hormone applications, see: Grover *et al.* (2007). For background to this study and previous syntheses, see: Shoppee *et al.* (1955). For related structures, see: Ketuly *et al.* (2011); Park (2004). For reference bond-length data, see: Allen *et al.* (1987). For the stability of the temperature controller used for the data collection, see: Cosier & Glazer (1986).



Experimental

Crystal data

$C_{27}H_{47}NO$
M_r = 401.65
Monoclinic, $P2_1$
a = 13.7535 (7) Å
b = 9.5266 (4) Å
c = 18.681 (1) Å
β = 102.829 (3)°
V = 2386.6 (2) Å^3
Z = 4
Mo $K\alpha$ radiation
μ = 0.07 mm^{-1}
T = 100 K
0.54 × 0.31 × 0.17 mm

Data collection

Bruker Kappa APEXII Duo diffractometer
Absorption correction: multi-scan (Blessing, 1995)
T_{min} = 0.965, T_{max} = 0.989
51587 measured reflections
7706 independent reflections
6021 reflections with $I > 2\sigma(I)$
R_{int} = 0.052

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.049
$wR(F^2)$ = 0.129
S = 1.12
7706 reflections
564 parameters
59 restraints
H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$ = 0.48 e Å^{-3}
$\Delta\rho_{min}$ = −0.32 e Å^{-3}

Data collection: *APEX2* (Bruker, 2008); cell refinement: *APEX2*; data reduction: *APEX2*; program(s) used to solve structure: *SHELXS97* (Sheldrick, 2008); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008); molecular graphics: *ORTEP-3* (Farrugia, 1997); software used to prepare material for publication: *publCIF* (Westrip, 2010).

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